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TITLE OF INVENTION

TRANSFERRIN RECEPTOR GENES OF MORAXELLA

FIELD OF INVENTION

The present invention relates to the molecular cloning of genes encoding transferrin receptor proteins and in particular to the cloning of transferrin receptor genes from *Moraxella (Branhamella) catarrhalis*.

REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of United States Patent Application No. 08/613,009 filed March 8, 1996.

BACKGROUND OF THE INVENTION

Moraxella (Branhamella) catarrhalis bacteria are Gram-negative diplococcal pathogens which are carried asymptomatically in the healthy human respiratory tract. In recent years, *M. catarrhalis* has been recognized as an important causative agent of otitis media. In addition, *M. catarrhalis* has been associated with sinusitis, conjunctivitis, and urogenital infections, as well as with a number of inflammatory diseases of the lower respiratory tract in children and adults, including pneumonia, chronic bronchitis, tracheitis, and emphysema (refs. 1 to 8). (Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). Occasionally, *M. catarrhalis* invades to cause septicaemia, arthritis, endocarditis, and meningitis (refs. 9 to 13).

Otitis media is one of the most common illnesses of

early childhood and approximately 80% of all children suffer at least one middle ear infection before the age of three (ref. 14). Chronic otitis media has been associated with auditory and speech impairment in children, and in some cases, has been associated with learning disabilities. Conventional treatments for otitis media include antibiotic administration and surgical procedures, including tonsillectomies, adenoidectomies, and tympanocentesis. In the United States, treatment costs for otitis media are estimated to be between one and two billion dollars per year.

In otitis media cases, *M. catarrhalis* commonly is co-isolated from middle ear fluid along with *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*, which are believed to be responsible for 50% and 30% of otitis media infections, respectively. *M. catarrhalis* is believed to be responsible for approximately 20% of otitis media infections (ref. 15). Epidemiological reports indicate that the number of cases of otitis media attributable to *M. catarrhalis* is increasing, along with the number of antibiotic-resistant isolates of *M. catarrhalis*. Thus, prior to 1970, no β-lactamase-producing *M. catarrhalis* isolates had been reported, but since the mid-seventies, an increasing number of β-lactamase-expressing isolates have been detected. Recent surveys suggest that 75% of clinical isolates produce β-lactamase (ref. 16, 26).

Iron is an essential nutrient for the growth of many bacteria. Several bacterial species, including *M. catarrhalis*, obtain iron from the host by using transferrin receptor proteins to capture transferrin. A number of bacteria including *Neisseria meningitidis* (ref. 17), *N. gonorrhoeae* (ref. 18), *Haemophilus influenzae* (ref. 19), as well as *M. catarrhalis* (ref. 20), produce outer membrane proteins which specifically bind human transferrin. The expression of these

proteins is regulated by the amount of iron in the environment.

The two transferrin receptor proteins of *M. catarrhalis*, designated transferrin binding protein 1 (Tbp1) and transferrin binding protein 2 (Tbp2), have molecular weights of 115 kDa (Tbp1) and approximately 80 to 90 kDa (Tbp2). Unlike the transferrin receptor proteins of other bacteria which have an affinity for apotransferrin, the *M. catarrhalis* Tbp2 receptors have a preferred affinity for iron-saturated (i.e., ferri-) transferrin (ref. 21).

M. catarrhalis infection may lead to serious disease. It would be advantageous to provide a recombinant source of transferrin binding proteins as antigens in immunogenic preparations including vaccines, carriers for other antigens and immunogens and the generation of diagnostic reagents. The genes encoding transferrin binding proteins and fragments thereof are particularly desirable and useful in the specific identification and diagnosis of *Moraxella* and for immunization against disease caused by *M. catarrhalis* and for the generation of diagnostic reagents.

SUMMARY OF THE INVENTION

The present invention is directed towards the provision of purified and isolated nucleic acid molecules encoding a transferrin receptor of a strain of *Moraxella* or a fragment or an analog of the transferrin receptor protein. The nucleic acid molecules provided herein are useful for the specific detection of strains of *Moraxella* and for diagnosis of infection by *Moraxella*. The purified and isolated nucleic acid molecules provided herein, such as DNA, are also useful for expressing the *tbp* genes by recombinant DNA means for providing, in an economical manner, purified and isolated transferrin receptor proteins as well as

subunits, fragments or analogs thereof. The transferrin receptor, subunits or fragments thereof or analogs thereof, as well as nucleic acid molecules encoding the same and vectors containing such nucleic acid molecules, are useful in immunogenic compositions for vaccinating against diseases caused by *Moraxella*, the diagnosis of infection by *Moraxella* and as tools for the generation of immunological reagents. Monoclonal antibodies or mono-specific antisera (antibodies) raised against the transferrin receptor protein produced in accordance with aspects of the present invention are useful for the diagnosis of infection by *Moraxella*, the specific detection of *Moraxella* (in, for example, *in vitro* and *in vivo* assays) and for the treatment of diseases caused by *Moraxella*.

In accordance with one aspect of the present invention, there is provided a purified and isolated nucleic acid molecule encoding a transferrin receptor protein of a strain of *Moraxella*, more particularly, a strain of *M. catarrhalis*, specifically *M. catarrhalis* strain 4223 or Q8, or a fragment or an analog of the transferrin receptor protein.

In one preferred embodiment of the invention, the nucleic acid molecule may encode only the Tbp1 protein of the *Moraxella* strain or only the Tbp2 protein of the *Moraxella* strain. In another preferred embodiment of the invention, the nucleic acid may encode a fragment of the transferrin receptor protein of a strain of *Moraxella* having an amino acid sequence which is conserved.

In another aspect of the present invention, there is provided a purified and isolated nucleic acid molecule having a DNA sequence selected from the group consisting of (a) a DNA sequence as set out in Figure 5, 6, 10 or 11 (SEQ ID Nos: 1, 2, 3, 4, 5, 6, 7 or 8) or the complementary DNA sequence of any one of said

sequences; (b) a DNA sequence ... sequence as set out in Figure 5, 6, 10 or 11 (SEQ ID Nos: 9, 10, 11, 12, 13, 14, 15 or 16) or the complementary DNA sequence thereto; and (c) a DNA sequence which hybridizes under stringent conditions to any one of the DNA sequences defined in (a) or (b). The DNA sequence defined in (c) preferably has at least about 90% sequence identity with any one of the DNA sequences defined in (a) and (b). The DNA sequence defined in (c) may be that encoding the equivalent transferrin receptor protein from another strain of *Moraxella*.

In an additional aspect, the present invention includes a vector adapted for transformation of a host, comprising a nucleic acid molecule as provided herein and may have the characteristics of a nucleotide sequence contained within vectors LEM3-24, pLEM3, pLEM25, pLEM23, SLRD-A, DS-1698-1-1, DS-1754-1, pSLRD1, pSLRD2, pSLRD3 and pSLRD4.

The vector may be adapted for expression of the encoded transferrin receptor, fragments or analogs thereof, in a heterologous or homologous host, in either a lipidated or non-lipidated form. Accordingly, a further aspect of the present invention provides an expression vector adapted for transformation of a host comprising a nucleic acid molecule as provided herein and expression means operatively coupled to the nucleic acid molecule for expression by the host of the transferrin receptor protein or the fragment or analog of the transferrin receptor protein. In specific embodiments of this aspect of the invention, the nucleic acid molecule may encode substantially all the transferrin receptor protein, only the Tbp1 protein, only the Tbp2 protein of the *Moraxella* strain or fragments of the Tbp1 or Tbp2 proteins. The expression means may include a promoter and a nucleic acid portion

encoding a leader sequence for secretion from the host of the transferrin receptor protein or the fragment or the analog of the transferrin receptor protein. The expression means also may include a nucleic acid portion encoding a lipidation signal for expression from the host of a lipidated form of the transferrin receptor protein or the fragment or the analog of the transferrin receptor protein. The host may be selected from, for example, *Escherichia coli*, *Bordetella*, *Bacillus*, *Haemophilus*, *Moraxella*, fungi, yeast or baculovirus and Semliki Forest virus expression systems may be used. In a particular embodiment the plasmid adapted for expression of Tbp1 is pLEM29 and that for expression of Tbp2 is pLEM33. Further vectors include pLEM-37, SLRD35-A and SLRD-35-B.

In an additional aspect of the invention, there is provided a transformed host containing an expression vector as provided herein. The invention further includes a recombinant transferrin receptor protein or fragment or analog thereof of a strain of *Moraxella* producible by the transformed host.

Such recombinant transferrin receptor protein may be provided in substantially pure form according to a further aspect of the invention, which provides a method of forming a substantially pure recombinant transferrin receptor protein, which comprises growing the transformed host provided herein to express a transferrin receptor protein as inclusion bodies, purifying the inclusion bodies free from cellular material and soluble proteins, solubilizing transferrin receptor protein from the purified inclusion bodies, and purifying the transferrin receptor protein free from other solubilized materials. The substantially pure recombinant transferrin receptor protein may comprise Tbp1 alone, Tbp2 alone or a mixture thereof. The recombinant protein is generally at least about 70%

pure, preferably at least about 90% pure.

Further aspects of the present invention, therefore, provide recombinantly-produced Tbp1 protein of a strain of *Moraxella* devoid of the Tbp2 protein of the *Moraxella* strain and any other protein of the *Moraxella* strain and recombinantly-produced Tbp2 protein of a strain of *Moraxella* devoid of the Tbp1 protein of the *Moraxella* strain and any other protein of the *Moraxella* strain. The *Moraxella* strain may be *M. catarrhalis* 4223 strain or *M. catarrhalis* Q8.

In accordance with another aspect of the invention, an immunogenic composition is provided which comprises at least one active component selected from at least one nucleic acid molecule as provided herein and at least one recombinant protein as provided herein, and a pharmaceutically acceptable carrier therefor or vector therefor. The at least one active component produces an immune response when administered to a host.

The immunogenic compositions provided herein may be formulated as vaccines for *in vivo* administration to a host. For such purpose, the compositions may be formulated as a microparticle, capsule, ISCOM or liposome preparation. The immunogenic composition may be provided in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces. The immunogenic compositions of the invention (including vaccines) may further comprise at least one other immunogenic or immunostimulating material and the immunostimulating material may be at least one adjuvant or at least one cytokine. Suitable adjuvants for use in the present invention include (but are not limited to) aluminum phosphate, aluminum hydroxide, QS21, Quil A, derivatives and components thereof, ISCOM matrix, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog, an octadecyl ester of an amino acid, a muramyl dipeptide

polyphosphazene, ISCOPREP, DC-chol, DDBA and a lipoprotein. Advantageous combinations of adjuvants are described in copending United States Patent Applications Nos. 08/261,194 filed June 16, 1994 and 08/483,856, filed June 7, 1995, assigned to the assignee hereof and the disclosures of which are incorporated herein by reference thereto.

In accordance with another aspect of the invention, there is provided a method for generating an immune response in a host, comprising the step of administering to a susceptible host, such as a human, an effective amount of the immunogenic composition provided herein. The immune response may be a humoral or a cell-mediated immune response and may provide protection against disease caused by *Moraxella*. Hosts in which protection against disease may be conferred include primates, including humans.

In a further aspect, there is provided a live vector for delivery of transferrin receptor to a host, comprising a vector containing the nucleic acid molecule as described above. The vector may be selected from *Salmonella*, BCG, adenovirus, poxvirus, vaccinia and poliovirus.

The nucleic acid molecules provided herein are useful in diagnostic applications. Accordingly, in a further aspect of the invention, there is provided a method of determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising the steps of:

(a) contacting the sample with a nucleic acid molecule as provided herein to produce duplexes comprising the nucleic acid molecule encoding the transferrin receptor protein of a strain of *Moraxella* present in the sample and specifically hybridizable therewith; and

(b) determining the production of the duplexes.

In addition, the present invention provides a diagnostic kit for determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising:

(a) a nucleic acid molecule as provided herein;

(b) means for contacting the nucleic acid molecule with the sample to produce duplexes comprising the nucleic acid molecule and any such nucleic acid present in the sample and hybridizable with the nucleic acid molecule; and

(c) means for determining production of the duplexes.

The invention further includes the use of the nucleic acid molecules and proteins provided herein as medicines. The invention additionally includes the use of the nucleic acid molecules and proteins provided herein in the manufacture of medicaments for protection against infection by strains of *Moraxella*.

Advantages of the present invention include:

- an isolated and purified nucleic acid molecule encoding a transferrin receptor protein of a strain of *Moraxella* or a fragment or an analog of the transferrin receptor protein;
- recombinantly-produced transferrin receptor proteins, including Tbp1 and Tbp2, free from each other and other *Moraxella* proteins; and
- diagnostic kits and immunological reagents for specific identification of *Moraxella*.

BRIEF DESCRIPTION OF DRAWINGS

The present invention will be further understood from the following description with reference to the drawings, in which:

Figure 1 shows the amino acid sequences (SEQ ID Nos: 17 and 18) used for synthesis of degenerate primers used for PCR amplification of a portion of the *M.*

catarrhalis 4223 *tbpA* gene;

Figure 2 shows a restriction map of clone LEM3-24 containing the *tbpA* and *tbpB* genes from *M. catarrhalis* isolate 4223;

Figure 3 shows a restriction map of the *tbpA* gene for *M. catarrhalis* 4223;

Figure 4 shows a restriction map of the *tbpB* gene for *M. catarrhalis* 4223;

Figure 5 shows the nucleotide sequence of the *tbpA* gene (SEQ ID No: 1 - entire sequence and SEQ ID No: 2 - coding sequence) and the deduced amino acid sequence of the Tbp1 protein from *M. catarrhalis* 4223 (SEQ ID No: 9 - full length and SEQ ID No: 10 - mature protein). The leader sequence (SEQ ID No: 19) is shown by underlining;

Figure 6 shows the nucleotide sequence of the *tbpB* gene (SEQ ID No: 3 - entire sequence and SEQ ID No: 4 - coding sequence) and the deduced amino acid sequence of the Tbp2 protein from *M. catarrhalis* 4223 (SEQ ID Nos: 11 - full length and SEQ ID No: 12 - mature protein). The leader sequence (SEQ ID No: 20) is shown by underlining;

Figure 7 shows a restriction map of clone SLRD-A containing the *tbpA* and *tbpB* genes from *M. catarrhalis* Q8;

Figure 8 shows a restriction map of the *tbpA* gene from *M. catarrhalis* Q8;

Figure 9 shows a restriction map of the *tbp*B gene from *M. catarrhalis* Q8;

Figure 10 shows the nucleotide sequence of the *tbpA* gene (SEQ. ID No: 5 - entire sequence and SEQ ID No: 6 - coding sequence) and the deduced amino acid sequence of the Tbp1 protein from *M. catarrhalis* Q8 (SEQ ID No: 13 - full length and SEQ ID No: 14 - mature protein);

Figure 11 shows the nucleotide sequence of the *tbpB* gene (SEQ. ID No: 7 - entire sequence and SEQ ID No: 8 - coding sequence) and the deduced amino acid sequence of

the Tbp2 protein from *M. catarrhalis* Q8 (SEQ ID No: 15 - full length and SEQ ID No: 16 - mature protein);

Figure 12 shows a comparison of the amino acid sequences of Tbp1 from *M. catarrhalis* strain 4223 (SEQ ID No: 9) and Q8 (SEQ ID No: 13), *H. influenzae* strain Eagan (SEQ ID No: 21), *N. meningitidis* strains B16B6 (SEQ ID No: 22) and M982 (SEQ ID No: 23) , and *N. gonorrhoeae* strain FA19 (SEQ ID No: 24);

Figure 13 shows a comparison of the amino acid sequences of Tbp2 from *M. catarrhalis* isolate 4223 (SEQ ID No: 11) and Q8 (SEQ ID No: 15), *H. influenzae* strain Eagan (SEQ ID No: 25), *N. meningitidis* strains B16B6 (SEQ ID No: 26) and M918 (SEQ ID No: 27), and *N. gonorrhoeae* strain FA19 (SEQ ID No: 28);

Figure 14 shows the construction of plasmid pLEM29 for expression of recombinant Tbp1 protein from *E. coli;*

Figure 15 shows the expression of Tbp1 protein by *E. coli* cells transformed with plasmid pLEM29;

Figure 16 shows a flow chart for purification of recombinant Tbp1 protein;

Figure 17 shows an SDS-PAGE analysis of purified recombinant Tbp1 protein;

Figure 18 shows the construction of plasmid pLEM33 and pLEM37 for expression of TbpA gene from *M. catarrhalis* 4223 in *E. coli* without and with a leader sequence respectively.

Figure 19 shows an SDS-PAGE analysis of the expression of rTbp2 protein by *E. coli* cells transformed with plasmid pLEM37;

Figure 20 shows the construction of plasmid sLRD35B for expression of the *tbpB* gene from *M. catarrhalis* Q8 in *E. coli* without a leader sequence, and the construction of plasmid SLRD35A for expression of the *tbpB* gene from *M. catarrhalis* Q8 in *E. coli* with a leader sequence. Restriction site B = BamHI; Bg = Bgl II; H = Hind III; R = EcoRI;

Figure 21 shows SDS PAGE analysis of the expression of rTbp2 protein in *E. coli* cell, transformed with plasmids SLRD35A and SLRD35B;

Figure 22 shows a flow chart for purification of recombinant Tbp2 protein from *E. coli*;

Figure 23, which includes Panels A and B, shows an SDS-PAGE analysis of the purification of recombinant Tbp2 protein from *M. catarrhalis* strains 4223 (Panel A) and Q8 (Panel B) for expression in *E. coli*;

Figure 24 shows the binding of Tbp2 to human transferrin; and

Figure 25, which includes Panels A, B and C, shows the antigenic conservation of Tbp2 protein amongst strains of *M. catarrhalis*.

GENERAL DESCRIPTION OF THE INVENTION

Any *Moraxella* strain may be conveniently used to provide the purified and isolated nucleic acid, which may be in the form of DNA molecules, comprising at least a portion of the nucleic acid coding for a transferrin receptor as typified by embodiments of the present invention. Such strains are generally available from clinical sources and from bacterial culture collections, such as the American Type Culture Collection.

In this application, the terms "transferrin receptor" (TfR) and "transferrin binding proteins" (Tbp) are used to define a family of Tbp1 and/or Tbp2 proteins which includes those having variations in their amino acid sequences including those naturally occurring in various strains of, for example, *Moraxella*. The purified and isolated DNA molecules comprising at least a portion coding for transferrin receptor of the present invention also include those encoding functional analogs of transferrin receptor proteins Tbp1 and Tbp2 of *Moraxella*. In this application, a first protein is a "functional analog" of a second protein if the first

protein is immunologically related to and/or has the same function as the second protein. The functional analog may be, for example, a fragment of the protein, or a substitution, addition or deletion mutant thereof.

Chromosomal DNA from *M. catarrhalis* 4223 was digested with *Sau*3A in order to generate fragments within a 15 to 23 kb size range, and cloned into the *Bam*HI site of the lambda vector EMBL3. The library was screened with anti-Tbp1 guinea pig antisera, and a positive clone LEM3-24, containing an insert approximately 13.2 kb in size was selected for further analysis. Lysate from *E. coli* LE392 infected with LEM3-24 was found to contain a protein approximately 115 kDa in size, which reacted on Western blots with anti-Tbp1 antisera. A second protein, approximately 80 kDa in size, reacted with the anti-Tbp2 guinea pig antisera on Western blots.

In order to localize the *tbpA* gene on the 13.2 kb insert of LEM3-24, degenerate PCR primers were used to amplify a small region of the putative tbpA gene of *M. catarrhalis* 4223. The sequences of the degenerate oligonucleotide primers were based upon conserved amino acid sequences within the Tbp1 proteins of several *Neisseria* and *Haemophilus* species Figure 1 (SEQ ID Nos: 17 and 18). A 300 base-pair amplified product was generated and its location within the 4223 *tbpA* gene is indicated by bold letters in Figure 5 (SEQ ID No: 29). The amplified product was subcloned into the vector pCRII, labelled, and used to probe a Southern blot containing restriction-endonuclease digested clone LEM3-24 DNA. The probe hybridized to a 3.8 kb *Hin*dIII-*Hin*dIII, a 2.0 kb *Avr*II-*Avr*II, and 4.2 kb *Sal*I-*Sph*I fragments (Figure 2).

The 3.8 kb *Hin*dIII-*Hin*dIII fragment was subcloned into pACYC177, and sequenced. A large open reading frame was identified, and subsequently found to contain

approximately 2 kb of the putative *tbpA* gene. The remaining 1 kb of the *tbpA* gene was obtained by subcloning an adjacent downstream *Hin*dIII-*Hin*dIII fragment into vector pACYC177. The nucleotide sequence of the *tbpA* gene from *M. catarrhalis* 4223 (SEQ ID No: 1), and the deduced amino acid sequence (SEQ ID No: 9) are shown in Figure 5.

Chromosomal DNA from *M. catarrhalis* strain Q8 was digested with Sau3A I and 15-23 kb fragments were ligated with BamH I arms of EMBL3. A high titre library was generated in *E. coli* LE392 cells and was screened using oligonucleotide probes based on the 4223 *tbpA* sequence. Phage DNA was prepared and restriction enzyme analysis revealed that inserts of about 13-15 kb had been cloned. Phage clone SLRD-A was used to subclone fragments for sequence analysis. A cloning vector (pSKMA) was generated to facilitate cloning of the fragments and plasmids pSLRD1, pSLRD2, pSLRD3, pSLRD4 and pSLRD5 were generated which contain all of *tbpA* and most of *tbpB*. The nucleotide (SEQ ID No: 5 and 6) and deduced amino acid sequence (SEQ ID No: 13 - full length, SEQ ID No: 14 - mature protein) of the *tbpA* gene from strain Q8 are shown in Figure 10.

The deduced amino acid sequences for the Tbp1 protein encoded by the *tbpA* genes were found to share some homology with the amino acid sequences encoded by genes from a number of *Neisseria* and *Haemophilus* species (Figure 12; SEQ ID Nos: 21, 22, 23 and 24).

Prior to the present discovery, *tbpA* genes identified in species of *Neisseria*, *Haemophilus*, and *Actinobacillus* have been found to be preceded by a *tbpB* gene with several conserved regions. The two genes typically are separated by a short intergenic sequence. However, a *tbpB* gene was not found upstream of the *tbpA* gene in *M. catarrhalis* 4223. In order to localize the *tbpB* gene within the 13.2 kb insert of clone LEM3-24, a

degenerate oligonucleotide probe was synthesized based upon an amino acid sequence EGGFYGP (SEQ ID No: 30), conserved among Tbp2 proteins of several species. The oligonucleotide was labelled and used to probe a Southern blot containing different restriction endonuclease fragments of clone LEM3-24. The probe hybridized to a 5.5 kb *Nhe*I-*Sal*I fragment, which subsequently was subcloned into pBR328, and sequenced. The fragment contained most of the putative *tbp*B gene, with the exception of the promoter region. The clone LEM3-24 was sequenced to obtain the remaining upstream sequence. The *tbp*B gene was located approximately 3 kb downstream from the end of the *tbp*A gene, in contrast to the genetic organization of the *tbp*A and *tbp*B genes in *Haemophilus* and *Neisseria*. The nucleotide sequence (SEQ ID No: 3) of the *tbp*B gene from *M. catarrhalis* 4223 and the deduced amino acid sequence (SEQ ID No: 11) are shown in Figure 6. The *tbpB* gene from *M. catarrhalis* Q8 was also cloned and sequenced. The nucleotide sequence (SEQ ID No: 7) and the deduced amino acid sequence (SEQ ID No: 15) are shown in Figure 11. Regions of homology are evident between the *M. catarrhalis* Tbp2 amino acid sequences and the Tbp2 sequences of a number of *Neisseria* and *Haemophilus* species, as shown in the comparative alignment in Figure 13 (SEQ ID Nos: 25, 26, 27, 28).

The cloned tbpA and tbpB genes were expressed in *E. coli* to produce recombinant Tbp1 and Tbp2 proteins free of other *Moraxella* proteins. These recombinant proteins were purified and used for immunization.

The antigenic conservation of Tbp2 protein amongst strains of *M. catarrhalis* was demonstrated by separation of the proteins in whole cell lysates of *M. catarrhalis* or strains of *E. coli* expressing recombinant Tbp2 proteins by SDS PAGE and antiserum immunoblotting with anti-4223 rTbp2 antiserum or anti-

Q8 rTbp2 antiserum raised in guinea pigs. *M. catarrhalis* strains 3, 56, 135, 585, 4223, 5191, 8185 and ATCC 25240 were tested in this way and all showed specific reactivity with anti-4223 rTbp2 or anti-Q8 rTbp2 antibody (Figure 26).

In addition, the ability of anti-rTbp2 antibodies from one strain to recognize native or recombinant protein from the homologous or heterologous strain by ELISA is shown in Table 1.

Amino acid sequencing of the N-termini and cyanogen bromide fragments of transferrin receptor from *M. catarrhalis* 4223 was undertaken. Both N-termini of Tbp1 and Tbp2 were blocked. The putative signal sequences of Tbp1 and Tbp2 are indicated by underlining in Figures 5 and 6 (SEQ ID Nos: 19 and 20) respectively. The deduced amino acid sequences for the N-terminal region of Tbp2 suggests a lipoprotein structure.

Results shown in Tables 1 and 2 below illustrate the ability of anti-Tbp1 and anti-Tbp2 guinea pig antisera, produced by the immunization with Tbp1 or Tbp2 to lyze *M. catarrhalis*. The results show that the antisera produced by immunization with Tbp1 or Tbp2 protein isolated from *M. catarrhalis* isolate 4223 were bactericidal against a homologous non-clumping *M. catarrhalis* strain RH408 (a strain previously deposited in connection with United States Patent Application No. 08/328,589, assigned to the assignee hereof, with the American Type Culture Collection, located at 1301 Parklawn Drive, Rockville, Maryland 20852, USA under the terms of the Budapest Treaty on December 13, 1994 under ATCC Deposit No. 55,637) derived from isolate 4223. In addition, antisera produced by immunization with Tbp1 protein isolated from *M. catarrhalis* 4223 were bactericidal against the heterologous non-clumping strain Q8 (a gift from Dr. M.G. Bergeron, Centre Hospitalier de l'Université Laval, St. Foy, Quebec). In

(rTbp2) protein was bacteriacidal against the homologous strain of *M. catarrhalis*.

The ability of isolated and purified transferrin binding protein to generate bactericidal antibodies is *in vivo* evidence of utility of these proteins as vaccines to protect against disease caused by *Moraxella*.

Thus, in accordance with another aspect of the present invention, there is provided a vaccine against *Moraxella* comprising an immunogenically-effective amount of transferrin binding protein and a physiologically-acceptable carrier therefor. Vaccine preparations may comprise antigenically or sequence divergent transferrin binding proteins. The transferrin binding protein provided herein may also be used as a carrier protein for haptens, polysaccharides or peptides to make conjugate vaccines against antigenic determinants unrelated to transferrin binding proteins.

The transferrin binding protein provided herein is useful as a diagnostic reagent, as an antigen or for the generation of anti-transferrin protein binding antibodies, antigen for vaccination against the disease caused by species of *Moraxella* and for detecting infection by *Moraxella* and other such bacteria.

In additional embodiments of the present invention, the transferrin binding protein as provided herein may be used as a carrier molecule to prepare chimeric molecules and conjugate vaccines (including glycoconjugates) against pathogenic bacteria, including encapsulated bacteria. Thus, for example, glycoconjugates of the present invention may be used to confer protection against disease and infection caused by any bacteria having polysaccharide antigens including lipooligosaccharides (LOS) and PRP. Such bacterial pathogens may include, for example, *Haemophilus influenzae, Streptococcus pneumoniae, Escherichia coli,*

Neisseria meningitidis, *Salmonella typhi*, *Streptococcus mutans*, *Cryptococcus neoformans*, *Klebsiella*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Particular antigens which can be conjugated to transferrin binding protein and methods to achieve such conjugations are described in published PCT application WO 94/12641, assigned to the assignee hereof and the disclosure of which is hereby incorporated by reference thereto.

In another embodiment, the carrier function of transferrin binding protein may be used, for example, to induce an immune response against abnormal polysaccharides of tumour cells, or to produce anti-tumour antibodies that can be conjugated to chemotherapeutic or bioactive agents.

The invention extends to transferrin binding proteins from *Moraxella catarrhalis* for use as a pharmaceutical substance as an active ingredient in a vaccine against disease caused by infection with *Moraxella*. The invention also extends to a pharmaceutical vaccinal composition containing transferrin binding proteins from *Moraxella catarrhalis* and optionally, a pharmaceutically acceptable carrier and/or diluent.

In a further aspect the invention provides the use of transferrin binding proteins for the preparation of a pharmaceutical vaccinal composition for immunization against disease caused by infection with *Moraxella*.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, treatment of, for example, *Moraxella* infections and the generation of immunological and other diagnostic reagents. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from immunogenic transferrin receptor proteins, analogs and fragments thereof encoded by the nucleic acid molecules as well as the nucleic acid molecules disclosed herein. The vaccine elicits an immune response which produces antibodies, including anti-transferrin receptor antibodies and antibodies that are opsonizing or bactericidal. Should the vaccinated subject be challenged by *Moraxella*, the antibodies bind to the transferrin receptor and thereby prevent access of the bacteria to an iron source which is required for viability. Furthermore, opsonizing or bactericidal anti-transferrin receptor antibodies may also provide protection by alternative mechanisms.

Immunogenic compositions including vaccines may be prepared as injectables, as liquid solutions or emulsions. The transferrin receptor proteins, analogs and fragments thereof and encoding nucleic acid molecules may be mixed with pharmaceutically acceptable excipients which are compatible with the transferrin receptor proteins, fragments, analogs or nucleic acid molecules. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness of the vaccines. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intradermally or intramuscularly. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. The immunogenic composition may

be provided in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces. Some such targeting molecules include vitamin B12 and fragments of bacterial toxins, as described in WO 92/17167 (Biotech Australia Pty. Ltd.), and monoclonal antibodies, as described in U.S. Patent No. 5,194,254 (Barber et al). Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 1 to 95% of the transferrin receptor proteins, fragments, analogs and/or nucleic acid molecules.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the transferrin receptor proteins, analogs and fragments thereof and/or nucleic acid molecules. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage of

the vaccine may also depend on the route of administration and will vary according to the size of the host.

The nucleic acid molecules encoding the transferrin receptor of *Moraxella* may be used directly for immunization by administration of the DNA directly, for example, by injection for genetic immunization or by constructing a live vector such as *Salmonella*, BCG, adenovirus, poxvirus, vaccinia or poliovirus. A discussion of some live vectors that have been used to carry heterologous antigens to the immune system are discussed in, for example, O'Hagan (ref 22). Processes for the direct injection of DNA into test subjects for genetic immunization are described in, for example, Ulmer et al. (ref. 23).

Immunogenicity can be significantly improved if the antigens are co-administered with adjuvants, commonly used as an 0.05 to 1.0 percent solution in phosphate - buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune

response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established and an HBsAg vaccine has been adjuvanted with alum. While the usefulness of alum is well established for some applications, it has limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

To efficiently induce humoral immune responses (HIR) and cell-mediated immunity (CMI), immunogens are often emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytolysis (saponins and pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

Desirable characteristics of ideal adjuvants

include:

(1) lack of toxicity;

(2) ability to stimulate a long-lasting immune response;

(3) simplicity of manufacture and stability in long-term storage;

(4) ability to elicit both CMI and HIR to antigens administered by various routes, if required;

(5) synergy with other adjuvants;

(6) capability of selectively interacting with populations of antigen presenting cells (APC);

(7) ability to specifically elicit appropriate T_H1 or T_H2 cell-specific immune responses; and

(8) ability to selectively increase appropriate antibody isotype levels (for example, IgA) against antigens.

US Patent No. 4,855,283 granted to Lockhoff et al on August 8, 1989 which is incorporated herein by reference thereto teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Thus, Lockhoff et al. 1991 (ref. 24) reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids, such as glycophospholipids and glycoglycerolipids, are capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine. Some glycolipids have been synthesized from long chain-alkylamines and fatty acids that are linked directly with the sugars through the anomeric carbon atom, to mimic the functions of the naturally occurring lipid residues.

U.S. Patent No. 4,258,029 granted to Moloney, assigned to the assignee hereof and incorporated herein by reference thereto, teaches that octadecyl tyrosine

hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus vaccine. Also, Nixon-George et al. 1990, (ref. 25) reported that octadecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen, enhanced the host immune responses against hepatitis B virus.

2. Immunoassays

The transferrin receptor proteins, analogs and/or fragments thereof of the present invention are useful as immunogens, as antigens in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art for the detection of anti-*Moraxella*, transferrin receptor protein antibodies. In ELISA assays, the transferrin receptor protein, analogs and/or fragments corresponding to portions of TfR protein, are immobilized onto a selected surface, for example, a surface capable of binding proteins or peptides such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed transferrin receptor, analogs and/or fragments, a non-specific protein such as a solution of bovine serum albumin (BSA) or casein that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by non-specific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as BSA, bovine gamma globulin (BGG) and/or phosphate buffered

saline (PBS)/Tween. The sample is then allowed to incubate for from 2 to 4 hours, at temperatures such as of the order of 25° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution such as PBS/Tween or a borate buffer.

Following formation of specific immunocomplexes between the test sample and the bound transferrin receptor protein, analogs and/or fragments and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having specificity for human immunoglobulins and in general IgG. To provide detecting means, the second antibody may have an associated activity such as an enzymatic activity that will generate, for example, a color development upon incubating with an appropriate chromogenic substrate. Quantification may then achieved by measuring the degree of color generation using, for example, a spectrophotometer.

3. Use of Sequences as Hybridization Probes

The nucleotide sequences of the present invention, comprising the sequence of the transferrin receptor gene, now allow for the identification and cloning of the transferrin receptor genes from any species of *Moraxella*.

The nucleotide sequences comprising the sequence of the transferrin receptor genes of the present invention are useful for their ability to selectively form duplex molecules with complementary stretches of other TfR genes. Depending on the application, a variety of hybridization conditions may be employed to achieve varying degrees of selectivity of the probe toward the

other TfR genes. For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt and/or high temperature conditions, such as provided by 0.02 M to 0.15 M NaCl at temperatures of between about 50°C to 70°C. For some applications, less stringent hybridization conditions are required such as 0.15 M to 0.9 M salt, at temperatures ranging from between about 20°C to 55°C. Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be a method of choice ~~depending on the desired results. In general,~~ convenient hybridization temperatures in the presence of 50% formamide are: 42°C for a probe which is 95 to 100% homologous to the target fragment, 37°C for 90 to 95% homology and 32°C for 85 to 90% homology.

In a clinical diagnostic embodiment, the nucleic acid sequences of the TfR genes of the present invention may be used in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin and digoxigenin-labelling, which are capable of providing a detectable signal. In some diagnostic embodiments, an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of a radioactive tag may be used. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with samples containing TfR gene sequences.

The nucleic acid sequences of TfR genes of the present invention are useful as hybridization probes in

solution hybridizations and in embodiments employing solid-phase procedures. In embodiments involving solid-phase procedures, the test DNA (or RNA) from samples, such as clinical samples, including exudates, body fluids (e. g., serum, amniotic fluid, middle ear effusion, sputum, bronchoalveolar lavage fluid) or even tissues, is adsorbed or otherwise affixed to a selected matrix or surface. The fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes comprising the nucleic acid sequences of the TfR genes or fragments thereof of the present invention under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required depending on, for example, the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe etc. Following washing of the hybridization surface so as to remove non-specifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label. It is preferred to select nucleic acid sequence portions which are conserved among species of *Moraxella*. The selected probe may be at least 18bp and may be in the range of about 30 to 90 bp.

4. Expression of the Transferrin Receptor Genes

Plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell may be used for the expression of the transferrin receptor genes in expression systems. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* may be transformed using pBR322 which contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid

or phage, must also contain, or be modified to contain, promoters which can be used by the host cell for expression of its own proteins.

In addition, phage vectors containing replicon and control sequences that are compatible with the host can be used as a transforming vector in connection with these hosts. For example, the phage in lambda GEM™-11 may be utilized in making recombinant phage vectors which can be used to transform host cells, such as *E. coli* LE392.

Promoters commonly used in recombinant DNA construction include the β-lactamase (penicillinase) and lactose promoter systems and other microbial promoters, such as the T7 promoter system as described in U.S. Patent No. 4,952,496. Details concerning the nucleotide sequences of promoters are known, enabling a skilled worker to ligate them functionally with genes. The particular promoter used will generally be a matter of choice depending upon the desired results. Hosts that are appropriate for expression of the transferrin receptor genes, fragments, analogs or variants thereof, may include *E. coli*, *Bacillus* species, *Haemophilus*, fungi, yeast, *Moraxella*, *Bordetella*, or the baculovirus expression system may be used.

In accordance with this invention, it is preferred to make the transferrin receptor protein, fragment or analog thereof, by recombinant methods, particularly when the naturally occurring TfR protein as purified from a culture of a species of *Moraxella* may include trace amounts of toxic materials or other contaminants. This problem can be avoided by using recombinantly produced TfR protein in heterologous systems which can be isolated from the host in a manner to minimize contaminants in the purified material. Particularly desirable hosts for expression in this regard include Gram positive bacteria which do not have LPS and are,

therefore, endotoxin free. Such hosts include species of *Bacillus* and may be particularly useful for the production of non-pyrogenic transferrin receptor, fragments or analogs thereof. Furthermore, recombinant methods of production permit the manufacture of Tbp1 or Tbp2 or analogs or fragments thereof separate from one another which is distinct from the normal combined proteins present in *Moraxella*.

Biological Deposits

Certain vectors that contain at least a portion coding for a transferrin receptor protein from strains of *Moraxella catarrhalis* strain 4223 and Q8 and a strain of *M. catarrhalis* RH408 that are described and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, USA, pursuant to the Budapest Treaty and prior to the filing of this application. Samples of the deposited vectors and bacterial strain will become available to the public and the restrictions imposed on access to the deposits will be removed upon grant of a patent based upon this United States patent application. In addition, the deposit will be replaced if viable samples cannot be dispensed by the Depository. The invention described and claimed herein is not to be limited in scope by the biological materials deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar vectors or strains that encode similar or equivalent antigens as described in this application are within the scope of the invention.

Deposit Summary

Deposit	ATCC Designation	Date Deposited
Phage LEM3-24	97,381	December 4, 1995
Phage SLRD-A	97,380	December 4, 1995
Plasmid pLEM29	97,461	March 8, 1996
Plasmid pSLRD35A		
Plasmid pLEM37		
Strain RH408	55,637	December 9, 1994

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example illustrates the preparation and immunization of guinea pigs with Tbp1 and Tbp2 proteins from *M. catarrhalis*.

Tbp1 and Tbp2 proteins were obtained as follows:

Iron-starved crude total membrane preparations were diluted to 4 mg protein/ml in 50 mM Tris.HCl-1M NaCl, pH 8, in a total volume of 384 ml. Membranes were solubilized by the addition of 8 ml each of 0.5M EDTA and 30% sarkosyl and samples were incubated for 2 hours at room temperature, with gentle agitation. Solubilized membranes were centrifuged at 10K rpm for 20 min. 15 ml of apo-hTf-Sepharose 4B were added to the supernatant, and incubated for 2 hours at room temperature, with gentle shaking. The mixture was added into a column. The column was washed with 50 ml of 50mM Tris.HCl-1 M NaCl-250mM guanidine hydrochloride, to remove contaminating proteins. Tbp2 was eluted from the column by the addition of 100 ml of 1.5M guanidine hydrochloride. Tbp1 was eluted by the addition of 100 ml of 3M guanidine hydrochloride. The first 20 ml fractions were dialyzed against 3 changes of 50 mM Tris.HCl, pH 8.0. Samples were stored at -20^{0}C, or dialyzed against ammonium bicarbonate and lyophilized.

Guinea pigs (Charles River) were immunized intramuscularly on day +1 with a 10 µg dose of Tbp1 or Tbp2 emulsified in complete Freund's adjuvant. Animals were boosted on days +14 and +29 with the same dose of protein emulsified in incomplete Freund's adjuvant. Blood samples were taken on day +42, and sera were used for analysis of bactericidal antibody activity. In addition, all antisera were assessed by immunoblot analysis for reactivity with *M. catarrhalis* 4223 proteins.

The bactericidal antibody activity of guinea pig anti-*M. catarrhalis* 4223 Tbp1 or Tbp2 antisera was determined as follows. A non-clumping *M. catarrhalis* strain RH408, derived from isolate 4223, was inoculated into 20 ml of BHI broth, and grown for 18 hr at 37°C, shaking at 170 rpm. One ml of this culture was used to inoculate 20 ml of BHI supplemented with 25 mM

ethylenediamine-di-hydroxyphenylacetic acid (EDDA; Sigma). The culture was grown to an OD_{578} of 0.5. The cells were diluted 1:200,000 in 140 mM NaCl, 93mM $NaHCO_3$, 2mM Na barbiturate, 4mM barbituric acid, 0.5mM $MgCl_2.6H_2O$, 0.4mM $CaCl_2.2H_2O$, pH 7.6 (Veronal buffer), containing 0.1% bovine serum albumin (VBS) and placed on ice. Guinea pig anti-*M. catarrhalis* 4223 Tbp1 or Tpb2 antisera, along with prebleed control antisera, were heated to 56°C for 30 min. to inactivate endogenous complement. Serial twofold dilutions of each antisera in VBS were added to the wells of a 96-well Nunclon microtitre plate (Nunc, Roskilde, Denmark). Dilutions started at 1:8, and were prepared to a final volume of 25 µL in each well. 25 µL of diluted bacterial cells were added to each of the wells. A guinea pig complement (Biowhittaker, Walkersville, MD) was diluted 1:10 in VBS, and 25 µL portions were added to each well. The plates were incubated at 37°C for 60 min, gently shaking at 70 rpm on a rotary platform. 50 µL of each reaction mixture were plated onto Mueller Hinton (Becton-Dickinson, Cockeysville, MD) agar plates. The plates were incubated at 37°C for 72 hr and the number of colonies per plate were counted. Bactericidal titres were assessed as the reciprocal of the highest dilution of antiserum capable of killing greater than 50% of bacteria compared with controls containing pre-immune sera. Results shown in Table 1 below illustrate the ability of the anti-Tbp1 and anti-Tbp2 guinea pig antisera to lyze *M. catarrhalis*.

Example 2

This Example illustrates the preparation of chromosomal DNA from *M. catarrhalis* strains 4223 and Q8.

M. catarrhalis isolate 4223 was inoculated into 100 ml of BHI broth, and incubated for 18 hr at 37°C with shaking. The cells were harvested by centrifugation at 10,000 x g for 20 min. The pellet was used for

extraction of *M. catarrhalis* 4223 chromosomal DNA.

The cell pellet was resuspended in 20 ml of 10 mM Tris-HCl (pH 7.5)-1.0 mM EDTA (TE). Pronase and SDS were added to final concentrations of 500 µg/ml and 1.0%, respectively, and the suspension was incubated at 37°C for 2 hr. After several sequential extractions with phenol, phenol:chloroform (1:1), and chloroform:isoamyl alcohol (24:1), the aqueous extract was dialysed, at 4°C, against 1.0 M NaCl for 4 hr, and against TE (pH 7.5) for a further 48 hr with three buffer changes. Two volumes of ethanol were added to the dialysate, and the DNA was spooled onto a glass rod. The DNA was allowed to air-dry, and was dissolved in 3.0 ml of water. Concentration was estimated, by UV spectrophotometry, to be about 290 µg/ml.

M. catarrhalis strain Q8 was grown in BHI broth as described in Example 1. Cells were pelleted from 50 ml of culture by centrifugation at 5000 rpm for 20 minutes, at 4°C. The cell pellet was resuspended in 10 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and proteinase K and SDS were added to final concentrations of 500 µg/ml and 1%, respectively. The sample was incubated at 37°C for 4 hours until a clear lysate was obtained. The lysate was extracted twice with Tris-saturated phenol/chloroform (1:1), and twice with chloroform. The final aqueous phase was dialysed for 24 hours against 2 X 1000 ml of 1 M NaCl at 4°C, changing the buffer once, and for 24 hours against 2 x 1000 ml of TE at 4°C, changing the buffer once. The final dialysate was precipitated with two volume of 100% ethanol. The DNA was spooled, dried and resuspended in 5 to 10 ml of TE buffer.

Example 3

This Example illustrates the construction of *M. catarrhalis* chromosomal libraries in EMBL3.

A series of *Sau*3A restriction digests of chromosomal DNA, in final volumes of 10 µL each, were

carri d out in order to optimize the conditions necessary to generate maximal amounts of restriction fragments within a 15 to 23 kb size range. Using the optimized digestion conditions, a large-scale digestion was set up in a 100 µL volume, containing the following: 50 µL of chromosomal DNA (290 µg/ml), 33 µL water, 10 µL 10X *Sau*3A buffer (New England Biolabs), 1.0 µL BSA (10 mg/mL, New England Biolabs), and 6.3 µL *Sau*3A (0.04 U/µL). Following a 15 min. incubation at 37°C, the digestion was terminated by the addition of 10 µL of 100 mM Tris-HCl (pH 8.0)-10 mM EDTA-0.1% bromophenol blue-50% glycerol (loading buffer). Digested DNA was electrophoresed through a 0.5% agarose gel in 40 mM Tris acetate-2 mM $Na_2EDTA.2H_2O$ (pH8.5) (TAE buffer) at 50 V for 6 hr. The region containing restriction fragments within a 15 to 23 kb molecular size range was excised from the gel, and placed into dialysis tubing containing 3.0 ml of TAE buffer. DNA was electroeluted from the gel fragment by applying a field strength of 1.0 V/cm for 18 hr. Electroeluted DNA was extracted once each with phenol and phenol:chloroform (1:1), and precipitated with ethanol. The dried DNA was dissolved in 5.0 µL water.

Size-fractionated chromosomal DNA was ligated with *Bam*HI-digested EMBL3 arms (Promega), using T4 DNA ligase in a final volume of 9 µL. The entire ligation mixture was packaged into lambda phage using a commercial packaging kit (Amersham), following manufacturer's instructions.

The packaged DNA library was amplified on solid media. 0.1 ml aliquots of *Escherichia coli* strain NM539 in 10 mM $MgSO_4$ (OD_{600} = 0.5) were incubated at 37°C for 15 min. with 15 to 25 µL of the packaged DNA library. Samples were mixed with 3 ml of 0.6% agarose containing 1.0% BBL trypticase peptone-0.5% NaCl (BBL top agarose), and mixtures were plated onto 1.5% agar plates

containing 1.0% BBL trypticase peptone-0.5% NaCl, and incubated at 37°C for 18 hr. 3 ml quantities of 50 mM Tris-HCl (pH 7.5)-8 mM magnesium sulfate heptahydrate-100 mM NaCl-0.01% (w/v) gelatin (SM buffer) were added to each plate, and plates were left at 4°C for 7 hr. SM buffer containing phage was collected from the plates, pooled together, and stored in a screwcap tube at 4°C, with chloroform.

Chromosomal DNA from *M. catarrhalis* strain Q8 was digested with Sau3A I (0.1 unit/30 µg DNA) at 37°C for 30 minutes and size-fractionated on a 0.6% low melting point agarose gel. DNA fragments of 15-23 kb were excised and the DNA was electroeluted for 25 minutes in dialysis tubing containing TAE (40 mM Tris acetate pH 8.5, 2 mM EDTA) at 150 V. The DNA was extracted once with phenol/chloroform (1:1), precipitated, and resuspended in water. The DNA was ligated overnight with EMBL3 BamH I arms (Promega) and the ligation mixture was packaged using the Lambda *in vitro* packaging kit (Stratagene) and plated onto *E. coli* LE392 cells. The library was titrated and stored at 4°C in the presence of 0.3% chloroform.

Example 4

This Example illustrates screening of the *M. catarrhalis* libraries.

Ten µL aliquots of phage stock from the EMBL3/4223 sample prepared in Example 3 above were combined each with 100 µL of *E. coli* strain LE392 in 10 mM $MgSO_4$ (OD_{260} = 0.5) (plating cells), and incubated at 37°C for 15 min. The samples were mixed with 3 ml each of BBL top agarose, and the mixtures were poured onto 1.5% agarose plates containing 1% bacto tryptone-0.5% bacto yeast extract-0.05% NaCl (LB agarose; Difco) and supplemented with 200 µM EDDA. The plates were incubated at 37°C for 18 hr. Plaques were lifted onto nitrocellulose filters (Amersham Hybond-C Extra) using a standard

protocol, and the filters were immersed into 5% bovine serum albumin (BSA; Boehringer) in 20 mM Tris-HCl (pH 7.5)-150 mM NaCl (TBS) for 30 min at room temperature, or 4°C overnight. Filters were incubated for at least 1 hr at room temperature, or 18 hr at 4°C, in TBS containing a 1/1000 dilution of guinea pig anti-*M. catarrhalis* 4223 Tbp1 antiserum. Following four sequential 10 min. washes in TBS with 0.05% Tween 20 (TBS-Tween), filters were incubated for 30 min. at room temperature in TBS-Tween containing a 1/4000 dilution of recombinant Protein G labelled with horseradish peroxidase (rProtein G-HRP; Zymed). Filters were washed as above, and submerged into CN/DAB substrate solution (Pierce). Color development was arrested by immersing the filters into water. Positive plaques were cored from the plates, and each placed into 0.5 ml of SM buffer containing a few drops of chloroform. The screening procedure was repeated two more times, until 100% of the lifted plaques were positive using the guinea pig anti-*M. catarrhalis* 4223 Tbp1 antiserum.

The EMBL3/Q8 library was plated onto LE392 cells on YT plates using 0.7% top agar in YT as overlay. Plaques were lifted onto nitrocellulose filters and the filters were probed with oligonucleotide probes labelled with $^{32}P\alpha$-dCTP (Random Primed DNA labeling kit, Boehringer Mannheim). The pre-hybridization was performed in sodium chloride/sodium citrate (SSC) buffer (ref. 27) at 37°C for 1 hour and the hybridization was performed at 42°C overnight. The probes were based upon an internal sequence of 4223 *tbpA*:

I R D L T R Y D P G

(Seq ID No. 31)

4236-RD 5' ATTCGAGACTTAACACGCTATGACCCTGGC 3'

(Seq ID No 32)

4237-RD 5' ATTCGTGATTTAACTCGCTATGACCCTGGT 3'

Putative plaques were re-plated and submitted to second and third rounds of screening using the same procedures. Phage clone SLRD-A was used to subclone the *tfr* genes for sequence analysis.

Example 5

This Example illustrates immunoblot analysis of the phage lysates using anti-*M. catarrhalis* 4223 Tbp1 and Tbp2 antisera.

Proteins expressed by the phage eluants selected in Example 4 above were precipitated as follows. 60 µL of each phage eluant were combined with 200 µL *E. coli* LE392 plating cells, and incubated at 37°C for 15 min. The mixture was inoculated into 10 ml of 1.0% NZamine A-0.5% NaCl-0.1% casamino acids-0.5% yeast extract-0.2% magnesium sulfate heptahydrate (NZCYM broth), supplemented with 200 mM EDDA, and grown at 37°C for 18 hr, with shaking. DNAse was added to 1.0 ml of the culture, to a final concentration of 50 µg/ml, and the sample was incubated at 37°C for 30 min. Trichloroacetic acid was added to a final concentration of 12.5%, and the mixture was left on ice for 15 min. Proteins were pelleted by centrifugation at 13,000 x g for 10 min, and the pellet was washed with 1.0 ml of acetone. The pellet was air-dried and resuspended in 50 µL 4% SDS-20 mM Tris- HCl (pH 8.0)-0.2 mM EDTA (lysis buffer).

Following SDS-PAGE electrophoresis through an 11.5% gel, the proteins were transferred to Immobilon-P filters (Millipore) at a constant voltage of 20 V for 18 hr, in 25mM Tris-HCl,220mM glycine-20% methanol (transfer buffer). Membranes were blocked in 5% BSA in TBS for 30 min. at room temperature. Blots were exposed either to guinea pig anti-*M. catarrhalis* 4223 Tbp1, or to guinea pig anti-*M. catarrhalis* 4223 Tbp2 antiserum,

diluted 1/500 in TBS-Tween, for 2 hr at room temperature. Following three sequential 10 min. washes in TBS-Tween, membranes were incubated in TBS-Tween containing a 1/4000 dilution of rProtein G-HRP for 30 min. at room temperature. Membranes were washed as described above, and immersed into CN/DAB substrate solution. Color development was arrested by immersing blots into water.

Three EMBL3 phage clones expressed both a 115 kDa protein which reacted with anti-Tbp1 antiserum, and an 80 kDa protein, which reacted with anti-Tbp2 antiserum on Western blots and were thus concluded to contain genes encoding the transferrin receptor proteins of *Moraxella catarrhalis*.

Example 6

This Example illustrates the subcloning of the *M. catarrhalis* 4223 Tbp1 protein gene, *tbpA*.

Plate lysate cultures of the recombinant phage described in Example 5 were prepared by combining phage eluant and *E. coli* LE392 plating cells, to produce confluent lysis on LB agar plates. Phage DNA was extracted from the plate lysates using a Wizard Lambda Preps DNA Purification System (Promega), according to manufacturer's instructions.

The EMBL3 clone LM3-24 was found to contain a 13.2 kb insert, flanked by two *Sal*I sites. A probe to a *tbpA* gene was prepared and consisted of a 300 base pair amplified product generated by PCR using two degenerate oligonucleotide primers corresponding to an amino acid sequence of part of the Tbp1 protein (Figure 1). The primer sequences were based upon the amino acid sequences NEVTGLG (SEQ ID No: 17) and GAINEIE (SEQ ID No: 18), which had been found to be conserved among the deduced amino acid sequences from several different *N. meningitidis* and *Haemophilus influenzae tbpA* genes. The amplified product was cloned into pCRII (Invitrogen, San

Diego, CA) and sequenced. The deduced amino acid sequence shared homology with other putative amino acid sequences derived from *N. meningitidis* and *H. influenzae* *tbpA* genes (Figure 12). The subclone was linearized with *Not*I (New England Biolabs), and labelled using a digoxigenin random-labelling kit (Boehringer Mannheim), according to manufacturer's instructions. The concentration of the probe was estimated to be 2 ng/μL.

DNA from the phage clone was digested with *Hin*dIII, *Avr*II, *Sal*I/*Sph*I, or *Sal*I/*Avr*II, and electrophoresed through a 0.8% agarose gel. DNA was transferred to a nylon membrane (Genescreen Plus, Dupont) using an LKB VacuGene XL vacuum transfer apparatus (Pharmacia). Following transfer, the blot was air-dried, and pre-hybridized in 5X SSC-0.1% N-lauroylsarcosine-0.02% sodium dodecyl sulfate-1.0% blocking reagent (Boehringer Mannheim) in 10 mM maleic acid-15 mM NaCl (pH 7.5) (pre-hybridization solution). Labelled probe was added to the pre-hybridization solution to a final concentration of 6 ng/ml, and the blot was incubated in the probe solution at 42°C for 18 hr. The blot was washed twice in 2X SSC-0.1% SDS, for 5 min. each at room temperature, then twice in 0.1X SSC-0.1% SDS for 15 min. each at 60°C. Following the washes, the membrane was equilibrated in 100mM maleic acid-150 mM NaCl (pH 7.5) (buffer 1) for 1 min, then left in 1.0% blocking reagent (Boehringer Mannheim) in buffer 1 (buffer 2) for 60 min, at room temperature. The blot was exposed to anti-DIG-alkaline phosphatase (Boehringer Mannheim) diluted 1/5000 in buffer 2, for 30 min. at room temperature. Following two 15 min. washes in buffer 1, the blot was equilibrated in 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM $MgCl_2$ (buffer 3) for 2 min. The blot was wetted with Lumigen PPD substrate (Boehringer- Mannheim), diluted 1/100 in buffer 3, then wrapped in Saran wrap, and exposed to X-ray film for 30 min. The probe

hybridized to a 3.8 kb *Hin*dIII-*Hin*dIII, a 2.0 kb *Avr*II-*Avr*II, and a 4.2 kb *Sal*I-*Sph*I fragment.

In order to subclone the 3.8 kb *Hin*dIII-*Hind*III fragment into pACYC177, phage DNA from the EMBL3 clone, and plasmid DNA from the vector pACYC177 (New England Biolabs), were digested with *Hin*dIII, and fractionated by electrophoresis on a 0.8% agarose gel. The 3.8 kb *Hin*dIII-*Hin*dIII phage DNA fragment, and the 3.9 kb *Hin*dIII-*Hin*dIII pACYC177 fragment, were excised from the gel and purified using a Geneclean kit (Bio 101 Inc., LaJolla, CA), according to manufacturer's directions. Purified insert and vector were ligated together using T4 DNA ligase (New England Biolabs), and transformed into *E. coli* HB101 (Gibco BRL). A Qiagen Plasmid Midi-Kit (Qiagen) was used to extract and purify sequencing-quality DNA from one of the ampicillin-resistant/kanamycin-sensitive transformants, which was found to carry a 3.8 kb *Hin*dIII-*Hin*dIII insert. The subclone was named pLEM3. As described in Example 7, below, subsequent sequencing revealed that pLEM3 contained the first about 2.0 kb of *tbpA* sequence (Figures 2 and 5).

In order to subclone the remaining 1 kb of the *tbpA* gene, a 1.6 kb *Hin*dIII-*Hin*dIII fragment was subcloned into pACYC177 as described above, and transformed by electroporation into *E. coli* HB101 (Gibco BRL). A Midi-Plasmid DNA kit (Qiagen) was used to extract plasmid DNA from a putative kanamycin-sensitive transformant carrying a plasmid with a 1.6 kb *Hin*dIII-*Hin*dIII insert. The subclone was termed pLEM25. As described in Example 7 below, sequencing revealed that pLEM25 contained the remaining 1 kb of the *tbpA* gene (Figure 2 and 5).

The *M. catarrhalis* Q8 tfr genes were subcloned as follows. Phage DNA was prepared from plates. Briefly, the top agarose layer from three confluent plates was

scraped into 9 ml of SM buffer (0.1 M NaCl, 0.2% $MgSO_4$, 50 mM Tris-HCl, pH 7.6, 0.01% gelatin) and 100 µl of chloroform was added. The mixture was vortexed for 10 sec, then incubated at room temperature for 2h. The cell debris was removed by centrifugation at 8000 rpm for 15 min at 4°C in an SS34 rotor (Sorvall model RC5C). The phage was pelleted by centrifugation at 35,000 rpm in a 70.1 Ti rotor at 10°C for 2h (Beckman model L8-80) and was resuspended in 500 µl of SM buffer. The sample was incubated at 4°C overnight, then RNAse and DNAse were added to final concentrations of 40 µg/ml and 10 µg/ml, respectively and the mixture incubated at 37°C for 1h. To the mixture were added 10 µl of 0.5 M EDTA and 5 µl of 10% SDS and the sample was incubated at 6°C for 15 min. The mixture was extracted twice with phenol/chloroform (1:1) and twice with chloroform and the DNA was precipitated by the addition of 2.5 volumes of absolute ethanol.

A partial restriction map was generated and fragments were subcloned using the external Sal I sites from EMBL3 and internal AvrII or EcoR I sites as indicated in Figure 4. In order to facilitate the subcloning, plasmid pSKMA was constructed which introduces a novel multiple cloning site into pBluescript.SK (Stratagene). Oligonucleotides were used to introduce restriction sites for Mst II, Sfi I, and Avr II between the Sal I and Hind III sites of pBluescript.SK:

```
                                  Sfi I
                  Sal I      Cla I    Mst II     Avr II HindIII
                    ↓          ↓        ↓    ↓    ↓       ↓
4639-RD    5' TCGACGGTAT CGATGGCC TTAG GGGC CTAGGA 3'
(SEQ ID No: 34)
4640-RD    3'      GCCATA GCTACCGG AATC CCCG GATCCTTCGA
(SEQ ID No: 31)
```

Plasmid pSLRD1 contains a ~1.5 kb Sal I-Avr II fragment cloned into pSKMA; plasmids pSLRD2 and pSLRD4 contain ~2 kb and 4 kb AvrII-AvrII fragments cloned into pSKMA, respectively. Plasmid pSLRD3 contains a ~2.3 kb AvrII-EcoR I fragment cloned into pSKMA and plasmid SLRD5 is a 22.7 kb EcoRI -EcoRI fragment cloned into pSKMA. These two clones contain the complete *tbpB* gene.

Example 7

This Example illustrates the subcloning of the *M. catarrhalis* 4223 *tbpB* gene.

As described above, in all *Neisseriae* and *Haemophilus* species examined prior to the present invention, *tbpB* genes have been found immediately upstream of the *tbpA* genes which share homology with the *tbpA* gene of *M. catarrhalis* 4223. However, the sequence upstream of *M. catarrhalis* 4223 did not correspond with other sequences encoding *tbpB*.

In order to localize the *tbpB* gene within the EMBL3 phage clone, a Southern blot was carried out using a degenerate probe from a highly conserved amino acid region within the Tbp2 protein. A degenerate oligonucleotide probe, was designed corresponding to the sequence encoding EGGFYGP (SEQ ID No: 30), which is conserved within the Tbp2 protein in a variety of *Neisseriae* and *Haemophilus* species. The probe was labelled with digoxigenin using an oligonucleotide tailing kit (Boehringer Mannheim), following the manufacturer's instructions. *Hin*dIII - digested EMBL3 clone DNA was fractionated through a 0.8% agarose gel, and transferred to a Geneclean Plus nylon membrane as described in Example 6. Following hybridization as described above, the membrane was washed twice in 2X SSC-0.1% SDS, for 5 min. each at room temperature, then twice in 0.1X SSC-0.1% SDS for 15 min. each, at 50°C. Detection of the labelled probe was carried out as

described above. The probe hybridized to a 5.5 kb *Nhe*I-*Sal*I fragment.

The 5.5 kb *Nhe*I-*Sal*I fragment was subcloned into pBR328 as follows. LEM3-24 DNA, and pBR328 DNA, were digested with *Nhe*I-*Sal*I, and electrophoresed through 0.8% agarose. The 5.5 kb *Nhe*I-*Sal*I fragment, and the 4.9 kb pBR328 *Nhe*I-*Sal*I fragments were excised from the gel, and purified using a Geneclean kit as described in Example 6. The fragments were ligated together using T4 DNA ligase, and transformed into *E. coli* DH5. A Midi-Plasmid DNA kit (Qiagen) was used to extract DNA from an ampicillin resistant / tetracycline sensitive clone containing a 5.5 kb *Nhe*I-*Sal*I insert. This subclone was termed pLEM23. Sequencing revealed that pLEM23 contained 2 kb of the *tbpB* gene from *M. catarrhalis* 4223 (Figure 2).

Example 8

This Example illustrates sequencing of the *M. catarrhalis tbp* genes.

Both strands of the *tbp* genes were sequenced using an Applied Biosystems DNA sequencer. The sequences of the *M. catarrhalis* 4223 and Q8 *tbpA* genes are shown in Figures 5 and 10 respectively. A derived amino acid sequence was compared with other Tbp1 amino acid sequences, including those of *Neisseriae meningitidis*, *Neisseriae gonorrhoeae*, and *Haemophilus influenzae* (Figure 12). The sequence of the *M. catarrhalis* 4223 and Q8 *tbpB* genes are shown in Figures 6 and 11 respectively. In order to obtain sequence from the putative beginning of the *tbpB* gene of *M. catarrhalis* 4223, sequence data were obtained directly from the clone LEM3-24 DNA. This sequence was verified by screening clone DS-1754-1. The sequence of the translated *tbpB* genes from *M. catarrhalis* 4223 and Q8 shared homology with deduced Tbp2 amino acid sequences of *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and

Haemophilus influenzae (Figure 13).

Example 9

This Example illustrates the generation of an expression vector to produce recombinant Tbp1 protein. The construction scheme is shown in Figure 14.

Plasmid DNA from subclone pLEM3 was digested with *Hin*dIII and *Bgl*I to generate a 1.84 kb *Bgl*I-*Hin*dIII fragment, containing approximately two-thirds of the *tbpA* gene. *Bam*HI was added to the digest to eliminate a comigrating 1.89kb *Bgl*I-*Hin*dIII vector fragment. In addition, plasmid DNA from the vector pT7-7 was digested with *Nde*I and *Hin*dIII. To create the beginning of the *tbpA* gene, an oligonucleotide was synthesized based upon the first 61 bases of the *tbpA* gene to the *Bgl*I site; an *Nde*I site was incorporated into the 5' end. Purified insert, vector and oligonucleotide were ligated together using T4 ligase (New England Biolabs), and transformed into *E. coli* DH5α. DNA was purified from one of the 4.4 kb ampicillin-resistant transformants containing correct restriction sites (pLEM27). Purified pLEM27 DNA was digested with *Hin*dIII, ligated to the 1.6 kb *Hin*dIII-*Hin*dIII insert fragment of pLEM25, and transformed into *E. coli* DH5α. DNA was purified from an ampicillin-resistant transformant containing the correct restriction sites (pLEM29), and was transformed by electroporation into BL21 (DE3) (Novagen; Madison, WI) to produce *E. coli* pLEM29B-1. A single isolated transformed colony was used to inoculate 100 ml of YT broth containing 100μg/ml ampicillin, and the culture was grown at 37^{0}C overnight, shaking at 200 rpm. 200 μl of the overnight culture were inoculated into 10 ml of YT broth containing 100μg/ml ampicillin, and the culture was grown at 37^{0}C to an OD_{578} of 0.35. The culture was induced by the addition of 30 μl of 100 mM IPTG, and the culture was grown at 37^{0}C for an additional 3

hours. One ml of culture was removed at the time of induction (t=0), and at t=1 hr and t=3 hrs. One ml samples were pelleted by centrifugation, and resuspended in 4%SDS-20 mM Tris.Cl, pH 8-200 µM EDTA (lysis buffer). Samples were fractionated on an 11.5% SDS-PAGE gel, and transferred onto Immobilon filters (Amersham). Blots were developed using anti-Tbp1 (*M. catarrhalis* 4223) antiserum, diluted 1:1000, as the primary antibody, and rproteinG conjugated with horseradish peroxidase (Zymed) as the secondary antibody. A chemiluminescent substrate (Lumiglo; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used for detection. Induced recombinant proteins were visible on the Coomassie-stained gels (Fig 15). The anti-Tbp1 (4223) antiserum recognized the recombinant proteins on Western blots.

Example 10

This Example illustrates the extraction and purification of recombinant Tbp1.

Recombinant Tbp1 protein was purified from *E. coli* cells expressing the tbpA gene as shown in Figure 16. *E. coli* cells from a 500 ml culture, prepared as described in Example 9, were resuspended in 50 ml of 50 mM Tris-HCl, pH 8.0 containing 0.1 M NaCl and 5 mM AEBSF (protease inhibitor), and disrupted by sonication (3 x 10 min. 70% duty circle). The extract was centrifuged at 20,000 x g for 30 min. and the resultant supernatant which contained > 85% of the soluble proteins from *E. coli* was discarded.

The remaining pellet (Figure 16, PPT1) was further extracted in 50 ml of 50 mM Tris, pH 8.0 containing 0.5% Triton X-100 and 10 mM EDTA. After centrifugation at 20,000 x g for 30 min., the supernatant containing residual soluble proteins and the majority of the membrane proteins was discarded.

The remaining pellet (Figure 16, PPT2) was further

extracted in 50 ml of 50 mM Tris, pH 8.0 containing 2M urea and 5 mM dithiothroitol (DTT). After centrifugation at 20,000 x g for 30 min., the resultant pellet (Figure 16, PPT3) obtained after the above extraction contained the inclusion bodies. The Tbp1 protein was solubilized from PPT-3 in 50 mM Tris, pH 8.0, containing 6 M guanidine hydrochloride and 5 mM DTT. After centrifugation, the resultant supernatant was further purified on a Superdex 200 gel filtration column equilibrated in 50 mM Tris, pH 8.0, containing 2M guanidine hydrochloride and 5 mM DTT. The fractions were analyzed by SDS-PAGE and those containing purified Tbp1 were pooled. Triton X-100 was added to the pooled Tbp1 fraction to a final concentration of 0.1%. The fraction was then dialyzed overnight at 4°C against 50 mM Tris, pH 8.0 and then centrifuged at 20,000 x g for 30 min. The protein remained soluble under these conditions and the purified Tbp1 was stored at -20° C. The purification procedure shown in Figure 16 produced Tbp1 protein that was at least 70% pure (Figure 17).

Example 11

This Example illustrates the construction of an expression plasmid for rTbp2 of *M. catarrhalis* 4223 without a leader sequence.

The construction scheme for rTbp2 is shown in Figure 18. Oligonucleotides were used to construct the first approximately 58 bp of the *M. catarrhalis* 4223 *tbpB* gene encoding the mature protein. An *Nde*I site was incorporated into the 5' end of the oligonucleotides:

5'TATGTGTGGTGGCAGTGGTGGTTCAAATCCACCTGCTCCTACGCCCATT CCAAATG (SEQ ID NO: 36) 3'

3'ACACACCACCGTCACCACCAAGTTTAGGTGGACGAGGATGCGGGTAAGG TTTACGATC (SEQ ID NO: 3) 5'

An *Nhe*I-*Cla*I fragment, containing approximately 1kb of the *tbpB* gene from pLEM23 was ligated to the above oligonucleotides and inserted into pT7-7 cut with *Nde*I-*Cla*I, generating pLEM31, which thus contains the 5'-half of *tbpB*. Oligonucleotides also were used to construct the last approximately 104 bp of the *tbpB* gene, from the *Ava*II site to the end of the gene. A *Bam*HI site was incorporated into the 3' end of the oligonucleotides:

5'GTCCAAATGCAAACGAGATGGGCGGGTCATTTACACACAACGCCGATG ACAGCAAAGCCTC

3'GTTTACGTTTGCTCTACCCGCCCAGTAAATGTGTGTTGCGGCTACTGTC GTTTCGGAG

TGTGGTCTTTGGCACAAAAAGACAACAAGAAGTTAAGTAGTAG (SEQ ID NO: 38) 3'

ACACCAGAAACCGTGTTTTTCTGTTGTTCTTCAATTCATCATCCTAG (SEQ ID NO: 39) 5'

A *Cla*I-*Ava*II fragment from pLEM23, containing approximately 0.9 kb of the 3'-end of the *tbpB* gene, was ligated to the *Ava*II-*Bam*HI oligonucleotides, and inserted into pT7-7 cut with *Cla*I-*Bam*HI, generating pLEM32. The 1.0 kb *Nde*I-*Cla*I insert from pLEM31 and the 1.0 kb *Cla*I-*Bam*HI insert from pLEM32 were then inserted into pT7-7 cut with *Nde*I-*Bam*HI, generating pLEM33 which has a full-length *tbpB* gene under the direction of the T7 promoter.

DNA was purified from pLEM33 and transformed by electroporation into electrocompetent BL21(DE3) cells (Novagen; Madison, WI), to generate strain pLEM33B-1. Strain pLEM33B-1 was grown, and induced using IPTG, as described above. Expressed proteins were resolved by SDS-PAGE and transferred to membranes suitable for immunoblotting. Blots were developed using anti-4223 Tbp2 antiserum, diluted 1:4000, as the primary antibody,

and rprotein G conjugated with horseradish peroxidase (Zymed) as the secondary antibody. A chemiluminescent substrate (Lumiglo; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used for detection. Induced recombinant proteins were visible on the Coomassie blue-stained gels (Fig. 19). The anti-4223 Tbp2 antiserum recognized the recombinant proteins on Western blots.

Example 12

This Example illustrates the generation of an expression plasmid for rTbp2 of *M. catarrhalis* 4223 with a leader sequence.

The construction scheme is shown in Figure 18. Oligonucleotides containing the natural leader sequence of the *M. catarrhalis* 4223 *tbpB* gene were used to construct the first approximately 115 bp of the *tbpB* gene to the *Nhe*I site. An *Nde*I site was incorporated into the 5' end of the oligonucleotides:

5'TATGAAACACATTCCTTTAACCACACTGTGTGTGGCAATCTCTGCCGTC TTATTAACCGCT

3'ACTTTGTGTAAGGAAATTGGTGTGACACACACCGTTAGAGACGGCAGAA TAATTGGCGA

TGTGGTGGCAGTGGTGGTTCAAATCCACCTGCTCCTACGCCCATTCCAAAT G (SEQ ID NO: 40) 3'

ACACCACCGTCACCACCAAGTTTAGGTGGACGAGGATGCGGGTAAGGTTTA CGATC (SEQ ID NO: 41) 5'

The *Nde*I-*Nhe*I oligonucleotides were ligated to pLEM33 cut with *Nde*I-*Nhe*I, generating pLEM37, which thus contains a full-length 4223 *tbpB* gene encoding the Tbp2 protein with its leader sequence, driven by the T7 promoter.

DNA from pLEM37 was purified and transformed by electroporation into electrocompetent BL21(DE3) cells (Novagen; Madison, WI), to generate strain pLEM37B-2. pLEM37B-2 was grown, and induced using IPTG, as

described above. Expressed proteins were resolved by SDS-PAGE and transferred to membranes suitable for immunoblotting. Blots were developed using anti-4223 Tbp2 antiserum, diluted 1:4000, as the primary antibody, and rprotein G conjugated with horseradish peroxidase (Zymed) as the secondary antibody. A chemiluminescent substrate (Lumiglo; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used for detection. Induced recombinant proteins were visible on Coomassie-blue stained gels (Fig. 21). The anti-4223 Tbp2 antiserum recognized the recombinant proteins on Western blots.

Example 13

This Example illustrates the construction of an expression plasmid for rTbp2 of *M. catarrhalis* Q8 without a leader sequence.

The construction scheme for rTbp2 is shown in Figure 20. The 5'-end of the *tbpB* gene of *M. catarrhalis* Q8 was PCR amplified from the Cys[1] codon of the mature protein through the Bsm I restriction site. An Nde I restriction site was introduced at the 5' end, for later cloning into pT7-7, and the final PCR fragment was 238 bp in length. The PCR primers are indicated below:

```
          NdeI   C   G   G   S   S   G   G   F   N
5' GAATTCCATATG TGT GGT GGG AGC TCT GGT GGT TTC AAT C
     3'  5247.RD (SEQ ID No: 42)
```

(CATATG underlined)

```
5' CCCATGGCAGGTTCTTGAATGCCTGAAACT        3'  5236.RD
(SEQ ID No: 43)
```

The 1.85 kb Bsm I-BamH I fragment from SLRD35 was ligated with the 238 bp PCR fragment and inserted into pT7-7 that had been digested with Nde I and BamH I, generating plasmid SLRD35B. This plasmid thus contains

the full-length *tbpB* gene without its leader sequence, under the direction of the T7 promoter. DNA from SLRD35B was purified and transformed by electroporation into electrocompetent BL21(DE3) cells to generate strain SLRD35BD which was grown and induced using IPTG, as described above. Expressed proteins were resolved by SDS-PAGE and the induced Tbp2 protein was clearly visible by Coomassie blue staining (Fig. 19).

Example 14

This Example illustrates the generation of an expression plasmid for rTbp2 of *M. catarrhalis* Q8 with a leader sequence.

The construction scheme for the rTbp2 is shown in Figure 20. The 5'-end of the Q8 *tbpB* gene was PCR amplified from the ATG start codon to the Bsm I restiction site. An Nde I site was engineered at the 5'-end, to facilitate cloning into the pT7-7 expression vector, and the final PCR fragment was 295 bp. The PCR primers are indicated below:

```
          Nde I   K   H   I   P   L   T
5' GAATTCCATATG AAA CAC ATT CCT TTA ACC 3'   5235.RD
(SEQ ID No: 42)

5' CCCATGGCAGGTTCTTGAATGCCTGAAACT       3'   5236.RD
(SEQ ID No: 43)
```

The Q8 *tbpB* gene was subcloned in two fragments contained on plasmids SLRD3 and SLRD5. Plasmid SLRD3-5 was constructed to contain the full-length *tbpB* gene by digesting SLRD5 with EcoR I and Dra I, which releases the 3'-end of *tbpB*, and inserting this ~ 619 bp fragment into SLRD3 which had been digested with EcoR I and Sma I. SLRD3-5 was digested with Bsm I and BamH I, generating a 1.85 kb fragment, which was ligated with the 295bp PCR fragment and ligated into pT7-7 that had

been digested with Nde I and BamH I. The resulting plasmid SLRD35A thus contains the full-length Q8 *tbpB* gene with its endogenous leader sequence under the control of the T7 promoter. DNA from SLRD35A was purified and transformed by electroporation into electrocompetent BL21(DE3) cells to generate strain SLRD35AD which was grown and induced using IPTG, as described above. Expressed proteins were resolved by SDS-PAGE and the induced Tbp2 protein was clearly visible by Coomassie blue staining (Fig. 19).

Example 15

This Example illustrates the extraction and purification of rTbp2 of *M. catarrhalis* 4223 and Q8 from *E. coli*.

pLEM37B (4223) and SLRD35AD (Q8) transformants were grown and then purified according to the scheme in Figure 22. *E. coli* cells from a 500 mL culture, were resuspended in 50 mL of 50 mM Tris-HCl, pH 8.0 containing 5 mM AEBSF (protease inhibitor), and disrupted by sonication (3 x 10 min, 70% duty circle). The extract was centrifuged at 20,000 x *g* for 30 min and the resultant supernatant which contained > 95% of the soluble proteins from *E. coli* was discarded.

The remaining pellet (PPT_1) was further extracted in 50 mL of 50 mM Tris, pH 8.0 containing 0.5% Triton X-100 and 10 mM EDTA. The mixture was stirred at 4°C for at least 2 hours and then centrifuged at 20,000 x *g* for 30 min and the supernatant containing residual soluble proteins and the majority of the membrane proteins was discarded.

The resultant pellet (PPT_2) obtained after the above extraction contained the inclusion bodies. The Tbp2 protein was solubilized in 50 mM Tris, pH 8.0, containing 6 M guanidine and 5 mM DTT. After centrifugation, the resultant supernatant was further

purified on a Superdex 200 gel filtration column equilibrated in 50 mM Tris, pH 8.0, containing 2 M guanidine and 5 mM DTT. The fractions were analyzed by SDS-PAGE and those containing purified Tbp2 were pooled. Triton X-100 was added to the pooled Tbp2 fraction to a final concentration of 0.1%. The fraction was then dialyzed overnight at 4°C against PBS, and then centrifuged at 20,000 x *g* for 30 min. The protein remained soluble under these conditions and the purified Tbp2 was stored at -20°C. Figure 22 shows the SDS PAGE analysis of fractions of the purification process for rTbp2 from strain 4223 (Panel A) and strain Q8 (Panel B). The rTbp2 was at least 70% pure.

Groups of five BALB/c mice were injected three times subcutaneously (s.c.) on days 1, 29 and 43 with purified rTbp2 (0.3 mg to 10 mg) from *M. catarrhalis* strains 4223 and Q8 in the presence or absence of $AlPO_4$ (1.5 mg per dose). Blood samples were taken on days 14, 28, 42 and 56 for analysing the anti-rTbp2 antibody titers by EIAs.

Groups of two rabbits and two guinea pigs (Charles River, Quebec) were immunized intramuscularly (i.m.) on day 1 with a 5 mg dose of purified rTbp2 protein emulsified in complete Freund's adjuvant (CFA). Animals were boosted on days 14 and 29 with the same dose of protein emulsified in incomplete Freund's adjuvant (IFA). Blood samples were taken on day 42 for analysing anti-rTbp2 antibody titers and bactericidal activity (Table 3).

Example 16

This Example illustrates the binding of Tbp2 to human transferrin *in vitro*.

Transferrin-binding activity of Tbp2 was assessed according to Schryvers and Lee (ref. 28) with modifications. Briefly, purified rTbp2 was subjected to discontinuous electrophoresis through 12.5% SDS-PAGE

gels. The proteins were electrophoretically transferred to PVDF membrane and incubated with horseradish peroxidase-conjugated human transferrin (HRP-human transferrin, 1:50 dilution) (Jackson ImmunoResearch Labs Inc., Mississauga, Ontario) at 4°C for overnight. LumiGLO substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was used for chemiluminescent detection of HRP activity according to the manufacturer's instructions. Both 4223 rTbp2 and Q8 rTbp2 bind to human transferrin under these conditions, as shown in Figure 24.

Example 17

This Example illustrates antigenic conservation of Tbp2 amongst *M. catarrhalis* strains.

Whole cell lysates of *M. catarrhalis* strains and *E. coli* strains expressing recombinant Tbp2 proteins were separated by SDS PAGE and electrophoretically transferred to PVDF membrane. Guinea pig anti-4223 rTbp2 or anti-Q8 rTbp2 antisera were used as first antibody and alkaline phosphatase conjugated goat anti-guinea pig antibody was used as second antibody to detect Tbp2. *M. catarrhalis* strains 3, 56, 135, 585, 4223, 5191, 8185 and ATCC 25240 were tested and all showed specific reactivity with anti-4223 rTbp2 or anti-Q8 rTbp2 antibody (Figure 25).

Table 3 illustrates the ability of anti-rTbp2 from one *M. catarrhalis* strain to recognize native or recombinant protein from a homologous or heterologous *M. catarrhalis* strain.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention provides purified and isolated DNA molecules containing transferrin receptor genes for *Moraxella catarrhalis*, the sequences of these transferrin receptor genes, and the derived amino acid sequences thereof.

The genes and DNA sequences are useful for diagnosis, immunization, and the generation of diagnostic and immunological reagents. Immunogenic compositions, including vaccines based upon expressed recombinant Tbp1 and/or Tbp2, portions thereof, or analogs thereof, can be prepared for prevention of diseases caused by Moraxella. Modifications are possible within the scope of this invention.

TABLE I

BACTERIAL ANTIBODY TITRES FOR
***M. CATARRHALIS* ANTIGENS**

ANTIGEN	SOURCE OF ANTISERA[2]	BACTERIAL TITRE[3] RH408[4]		BACTERIAL TITRE Q8[5]	
		Pre-Immune	Post-Immune	Pre-Immune	Post-Immune
TBP1	GP	< 3.0	4.2-6.9	< 3.0	4.4.-6.2
TBP2	GP	< 3.0	12.0-13.6	< 3.0	< 3.0-4.0

1 antigens isolated from *M. catarrhalis* 4223
2 GP = guinea pig
3 bacterial titres: expressed in $\log_2$ as the dilution of antiserum capable of killing 50% of cells
4 *M. catarrhalis* RH408 is a non-clumping derivative of 4223
5 *M. catarrhalis* Q8 is a clinical isolate which displays a non-clumping phenotype

TABLE 2

Bactericidal activity of antibodies raised to recombinant transferrin binding proteins

	Bactericidal titre - RH408		Bactericidal titre - Q8	
Antigen	pre-immune	post-immune	pre-immune	post-immune
rTbp1 (4223)	< 3.0	< 3.0	< 3.0	< 3.0
rTbp2 (4223)	< 3.0	10-15	< 3.0	< 3.0
rTbp2 (Q8)	NT	NT	< 3.0	5.5-7.5

Antibody titres are expressed in $\log_2$ as the dilution of antiserum capable of killing 50% of cells

NT = not tested

TABLE 3

ELISA titres for anti-rTbp2 antibodies recognizing native or rTbp2 from strain 4223 or rTbp2 from strain Q8

Coated antigen	Anti-rTbp2 (4223) Antibody Titres		Anti-rTbp2 (Q8) Antibody Titres	
	Rabbit antisera	Guinea pig antisera	Rabbit antisera	Guinea pig antisera
Native Tbp2 (4223)	409,600 204,800	1,638,400 1,638,400	25,600 25,600	51,200 102,400
rTbp2 (4223)	409,600 409,600	1,638,400 1,638,400	102,400 102,400	204,800 204,800
rTbp2 (Q8)	409,600 102,400	1,638,400 1,638,400	1,638,400 409,600	1,638,400 1,638,400

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What we claim is:

1. A purified and isolated nucleic acid molecule encoding a transferrin receptor protein of a strain of *Moraxella* or a fragment or an analog of the transferrin receptor protein.

2. The nucleic acid molecule of claim 1 wherein the transferrin receptor protein is the transferrin receptor binding protein 1 (Tbp1) of the *Moraxella* strain.

3. The nucleic acid molecule of claim 2 wherein the transferrin receptor protein is the transferrin receptor binding protein (Tbp2) of the *Moraxella* strain.

4. The nucleic acid molecule of claim 1 wherein the strain of *Moraxella* is a strain of *Moraxella catarrhalis*.

5. The nucleic acid molecule of claim 4 wherein the strain of *Moraxella catarrhalis* is *Moraxella catarrhalis* 4223 or Q8.

6. A purified and isolated nucleic acid molecule having a DNA sequence selected from the group consisting of:

(a) a DNA sequence as set out in Figure 5, 6, 10 or 11 (SEQ ID Nos: 1, 2, 3, 4, 5, 6, 7 or 8) or the complementary DNA sequence thereto;

(b) a DNA sequence encoding an amino acid sequence as set out in Figure 5, 6, 10 or 11 (SEQ ID Nos: 9, 10, 11, 12, 13, 14, 15 or 16) or the complementary DNA sequence thereto; and

(c) a DNA sequence which hybridizes under stringent conditions to any one of the DNA sequences defined in (a) or (b).

7. The nucleic acid molecule of claim 6, wherein the DNA sequence defined in (c) has at least about 90% sequence identity with any one of the DNA sequences defined in (a) or (b).

8. The nucleic acid molecule of claim 6 wherein the DNA sequence defined in (c) is that encoding the equivalent transferrin receptor protein from another strain of *Moraxella*.

9. A vector adapted for transformation of a host comprising the nucleic acid molecule of claim 1 or 6.

10. The vector of claim 9 encoding a fragment of a transferrin receptor protein and having the characteristics of a plasmid selected from the group consisting of pLEM3, pLEM25, pLEM23, DS-1698-1-1, DS-1754-1, pSLRD1, pSLRD2, pSLRD3, pSLRD4 and pSLRD5.

11. The vector of claim 9 further comprising expression means operatively coupled to the nucleic acid molecule for expression by the host of said transferrin receptor protein of a strain of *Moraxella* or the fragment or the analog of the transferrin receptor protein.

12. The vector of claim 11 having the characteristics of plasmid pLEM-29, pLEM-33, pLEM-37, SLRD35-A and SLRD35-B.

13. A transformed host containing an expression vector as claimed in claim 11.

14. A method of forming a substantially pure recombinant transferrin receptor protein, which comprises:

growing the transformed host of claim 13 to express a transferrin receptor protein as inclusion bodies,

purifying the inclusion bodies free from cellular material and soluble proteins,

solubilizing transferrin receptor protein from the purified inclusion bodies, and

purifying the transferrin receptor protein free from other solubilized materials.

15. The method of claim 14 wherein said transferrin

receptor protein comprises Tbp1 alone, Tbp2 alone or a mixture of Tbp1 and Tbp2.

16. The method of claim 15 wherein said transferrin receptor protein is at least about 70% pure.

17. The method of claim 16 wherein said transferrin receptor protein is at least about 90% pure.

18. A recombinant transferrin receptor protein or fragment or analog thereof producible by the transformed host of claim 12.

19. The protein of claim 18 which is transferrin receptor binding protein 1 (Tbp1) of the *Moraxella* strain devoid of other proteins of the *Moraxella* strain.

20. The protein of claim 18 which is transferrin receptor binding protein 2 (Tbp2) of the *Moraxella* strain devoid of other proteins of the *Moraxella* strain.

21. The protein of claim 18 wherein the strain of *Moraxella* is a strain of *Moraxella catarrhalis*.

22. An immunogenic composition, comprising at least one active component selected from the group consisting of:

(A) a purified and isolated nucleic acid molecule encoding a transferrin receptor protein of a strain of *Moraxella* or a fragment or an analog of the transferrin receptor protein;

(B) a purified and isolated nucleic acid molecule having a DNA sequence selected from the group consisting of:

(a) a DNA sequence as set out in Figure 5, 6, 10 or 11 (SEQ ID Nos: 1, 2, 3, 4, 5, 6, 7 or 8) or the complementary DNA sequence thereto;

(b) a DNA sequence encoding an amino acid sequence as set out in Figure 5, 6, 10 or 11 (SEQ ID Nos: 9, 10, 11, 12, 13, 14, 15 or 16) or the complementary DNA sequence thereto; and

(c) a DNA sequence which hybridizes under stringent conditions to any one of the DNA sequences defined in (a) or (b); or

(C) a recombinant transferrin receptor protein or fragment or analog thereof producible by a transformed host containing an expression vector comprising a nucleic acid molecule as defined in (A) or (B) and expression means operatively coupled to the nucleic acid molecule for expression by the host of the recombinant transferrin receptor protein or fragment or analog thereof;

and a pharmaceutically acceptable carrier therefor, said at least one active component producing an immune response when administered to a host.

23. A method for generating an immune response in a host, comprising administering to the host an immunoeffective amount of the immunogenic composition of claim 22.

24. A method of determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising the steps of:

(a) contacting the sample with the nucleic acid molecule of claim 1 or 6 to produce duplexes comprising the nucleic acid molecule and any said nucleic acid molecule encoding the transferrin receptor protein of a strain of *Moraxella* present in the sample and specifically hybridizable therewith; and

(b) determining production of the duplexes.

25. A diagnostic kit for determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising:

(a) the nucleic acid molecule of claim 1 or 6;

(b) means for contacting the nucleic acid molecule with the sample to produce duplexes comprising

the nucleic acid molecule and any said nucleic acid present in the sample and hybridizable with the nucleic acid molecule; and

(c) means for determining production of the duplexes.

ABSTRACT OF THE DISCLOSURE

Purified and isolated nucleic acid molecules are provided which encode transferrin receptor proteins of *Moraxella*, such as *M. catarrhalis* or a fragment or an analog of the transferrin receptor protein. The nucleic acid sequence may be used to produce recombinant transferrin receptor proteins Tbp1 and Tbp2 of the strain of *Moraxella* free of other proteins of the *Moraxella* strain for purposes of diagnostics and medical treatment. Furthermore, the nucleic acid molecule may be used in the diagnosis of infection.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: **TRANSFERRIN RECEPTOR GENES OF MORAXELLA**, the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, S.1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, S.119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
			Yes	No
(Number)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under Title 35, United States Code, S.120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, S.112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, S.1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

08/613,009	March 8, 1996	Pending
(Appln. Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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FIG. 1

AMINO ACID SEQUENCES OF A CONSERVED PORTION OF Tbp1 PROTEIN FOR CONSTRUCTION OF DEGENERATE PRIMERS USED IN PCR AMPLIFICATION OF A PORTION OF THE *M. cattarhalis* 4223 *tbp*A GENE.

N E V T G L G	SEQ ID NO: 17
G A I N E I E	SEQ ID NO: 18

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M. catarrhalis 4223 Transferrin Receptor Genes

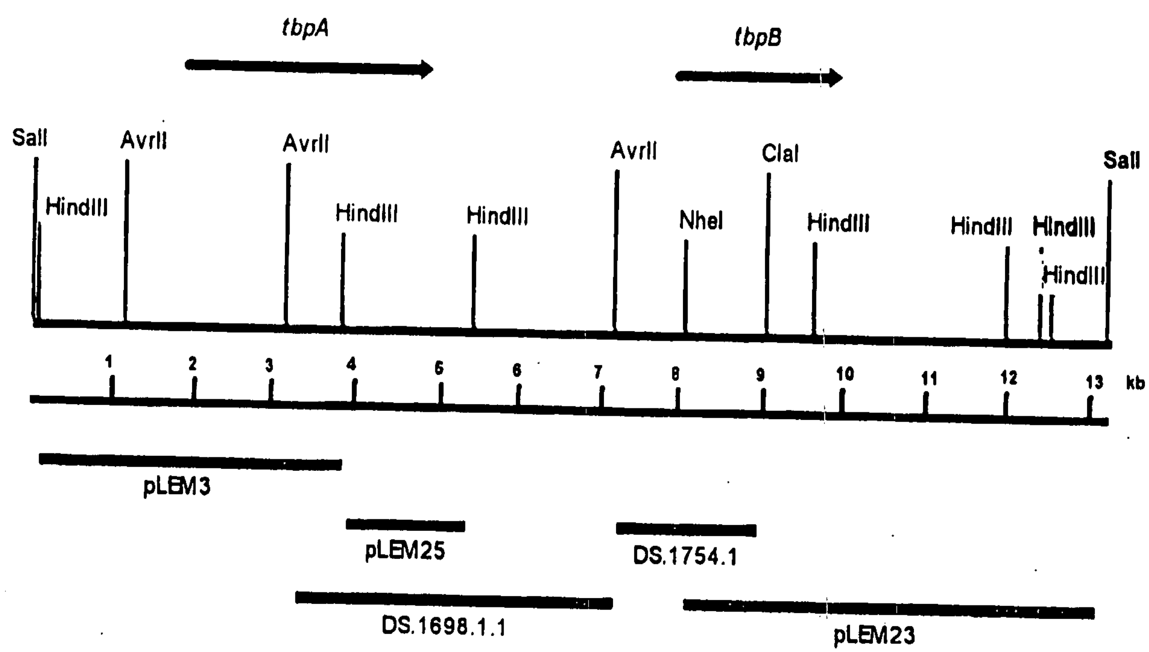

FIG. 2

M. catarrhalis 4223 *tbpA* gene

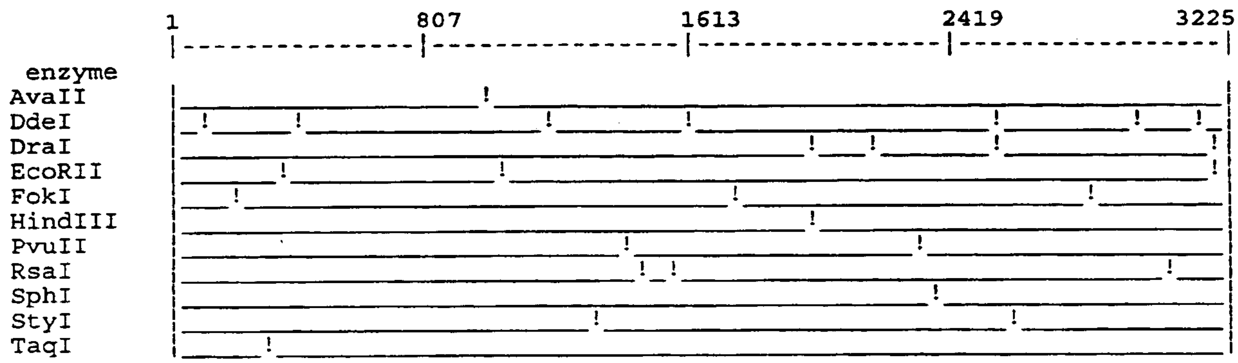

FIG. 3

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M. catarrhalis 4223 *tbpB* gene

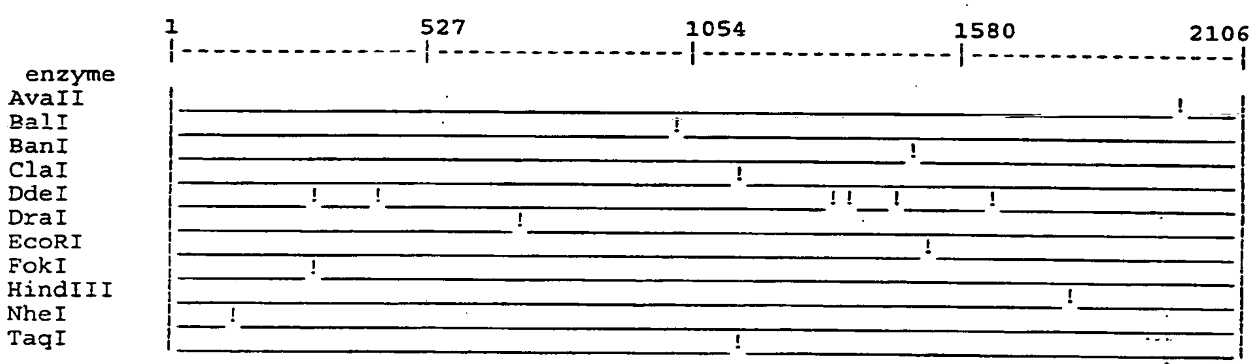

FIG. 4

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FIG. 5

Sequence of *M. catarrhalis* 4223 *tbpA* gene

TATTTTGACAAGCTATACACTAAAATCAAAAATTAATCACTTTGGTTGGGTGGTTTTAGCAAGCAAATGGT

TATTTTGGTAAACAATTAAGTTCTTAAAAACGATACACGCTCATAAACAGATGGTTTTTGGCATCTGCAAT

TTGATGCCTGCCTTGTGATTGGTTGGGGTGTATCGGTGTATCAAAGTGCAAAAGCCAACAGGTGGTCATTG

```
                              27                         54
ATG AAT CAA TCA AAA CAA AAC AAC AAA TCC AAA AAA TCC AAA CAA GTA TTA AAA
MET Asn Gln Ser Lys Gln Asn Asn Lys Ser Lys Lys Ser Lys Gln Val Leu Lys

                              81                         108
CTT AGT GCC TTG TCT TTG GGT CTG CTT AAC ATC ACG CAG GTG GCA CTG GCA AAC
Leu Ser Ala Leu Ser Leu Gly Leu Leu Asn Ile Thr Gln Val Ala Leu Ala Asn

                              135                        162
ACA ACG GCC GAT AAG GCG GAG GCA ACA GAT AAG ACA AAC CTT GTT GTT GTC TTG
Thr Thr Ala Asp Lys Ala Glu Ala Thr Asp Lys Thr Asn Leu Val Val Val Leu

                              189                        216
GAT GAA ACT GTT GTA ACA GCG AAG AAA AAC GCC CGT AAA GCC AAC GAA GTT ACA
Asp Glu Thr Val Val Thr Ala Lys Lys Asn Ala Arg Lys Ala Asn Glu Val Thr

                              243                        270
GGG CTT GGT AAG GTG GTC AAA ACT GCC GAG ACC ATC AAT AAA GAA CAA GTG CTA
Gly Leu Gly Lys Val Val Lys Thr Ala Glu Thr Ile Asn Lys Glu Gln Val Leu

                              297                        324
AAC ATT CGA GAC TTA ACA CGC TAT GAC CCT GGC ATT GCT GTG GTT GAG CAA GGT
Asn Ile Arg Asp Leu Thr Arg Tyr Asp Pro Gly Ile Ala Val Val Glu Gln Gly

                              351                        378
CGT GGG GCA AGC TCA GGC TAT TCT ATT CGT GGT ATG GAT AAA AAT CGT GTG GCG
Arg Gly Ala Ser Ser Gly Tyr Ser Ile Arg Gly MET Asp Lys Asn Arg Val Ala

                              405                        432
GTA TTG GTT GAT GGC ATC AAT CAA GCC CAG CAC TAT GCC CTA CAA GGC CCT GTG
Val Leu Val Asp Gly Ile Asn Gln Ala Gln His Tyr Ala Leu Gln Gly Pro Val
```

FIG. 5 Cont.

```
                                459                                 486
GCA GGC AAA AAT TAT GCC GCA GGT GGG GCA ATC AAC GAA ATA GAA TAC GAA AAT
Ala Gly Lys Asn Tyr Ala Ala Gly Gly Ala Ile Asn Glu Ile Glu Tyr Glu Asn

                                513                                 540
GTC CGC TCC GTT GAG ATT AGT AAA GGT GCA AAT TCA AGT GAA TAC GGC TCT GGG
Val Arg Ser Val Glu Ile Ser Lys Gly Ala Asn Ser Ser Glu Tyr Gly Ser Gly

                                567                                 594
GCA TTA TCT GGC TCT GTG GCA TTT GTT ACC AAA ACC GCC GAT GAC ATC ATC AAA
Ala Leu Ser Gly Ser Val Ala Phe Val Thr Lys Thr Ala Asp Asp Ile Ile Lys

                                621                                 648
GAT GGT AAA GAT TGG GGC GTG CAG ACC AAA ACC GCC TAT GCC AGT AAA AAT AAC
Asp Gly Lys Asp Trp Gly Val Gln Thr Lys Thr Ala Tyr Ala Ser Lys Asn Asn

                                675                                 702
GCA TGG GTT AAT TCT GTG GCA GCA GCA GGC AAG GCA GGT TCT TTT AGC GGT CTT
Ala Trp Val Asn Ser Val Ala Ala Ala Gly Lys Ala Gly Ser Phe Ser Gly Leu

                                729                                 756
ATC ATC TAC ACC GAC CGC CGT GGT CAA GAA TAC AAG GCA CAT GAT GAT GCC TAT
Ile Ile Tyr Thr Asp Arg Arg Gly Gln Glu Tyr Lys Ala His Asp Asp Ala Tyr

                                783                                 810
CAG GGT AGC CAA AGT TTT GAT AGA GCG GTG GCA ACC ACT GAC CCA AAT AAC CGA
Gln Gly Ser Gln Ser Phe Asp Arg Ala Val Ala Thr Thr Asp Pro Asn Asn Arg

                                837                                 864
ACA TTT TTA ATA GCA AAT GAA TGT GCC AAT GGT AAT TAT GAG GCG TGT GCT GCT
Thr Phe Leu Ile Ala Asn Glu Cys Ala Asn Gly Asn Tyr Glu Ala Cys Ala Ala

                                891                                 918
GGC GGT CAA ACC AAA CTT CAA GCC AAG CCA ACC AAT GTG CGT GAT AAG GTC AAT
Gly Gly Gln Thr Lys Leu Gln Ala Lys Pro Thr Asn Val Arg Asp Lys Val Asn

                                945                                 972
GTC AAA GAT TAT ACA GGT CCT AAC CGC CTT ATC CCA AAC CCA CTC ACC CAA GAC
Val Lys Asp Tyr Thr Gly Pro Asn Arg Leu Ile Pro Asn Pro Leu Thr Gln Asp

                                999                                1026
AGC AAA TCC TTA CTG CTT CGC CCA GGT TAT CAG CTA AAC GAT AAG CAC TAT GTC
Ser Lys Ser Leu Leu Leu Arg Pro Gly Tyr Gln Leu Asn Asp Lys His Tyr Val
```

FIG. 5 Cont.

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1053 1080
GGT GGT GTG TAT GAA ATC ACC AAA CAA AAC TAC GCC ATG CAA GAT AAA ACC GTG
Gly Gly Val Tyr Glu Ile Thr Lys Gln Asn Tyr Ala MET Gln Asp Lys Thr Val

1107 1134
CCT GCT TAT CTG ACG GTT CAT GAC ATT GAA AAA TCA AGG CTC AGC AAC CAT GCC
Pro Ala Tyr Leu Thr Val His Asp Ile Glu Lys Ser Arg Leu Ser Asn His Ala

1161 1188
CAA GCC AAT GGC TAT TAT CAA GGC AAT AAT CTT GGT GAA CGC ATT CGT GAT ACC
Gln Ala Asn Gly Tyr Tyr Gln Gly Asn Asn Leu Gly Glu Arg Ile Arg Asp Thr

1215 1242
ATT GGG CCA GAT TCA GGT TAT GGC ATC AAC TAT GCT CAT GGC GTA TTT TAT GAT
Ile Gly Pro Asp Ser Gly Tyr Gly Ile Asn Tyr Ala His Gly Val Phe Tyr Asp

1269 1296
GAA AAA CAC CAA AAA GAC CGC CTA GGG CTT GAA TAT GTT TAT GAC AGC AAA GGT
Glu Lys His Gln Lys Asp Arg Leu Gly Leu Glu Tyr Val Tyr Asp Ser Lys Gly

1323 1350
GAA AAT AAA TGG TTT GAT GAT GTG CGT GTG TCT TAT GAT AAG CAA GAC ATT ACG
Glu Asn Lys Trp Phe Asp Asp Val Arg Val Ser Tyr Asp Lys Gln Asp Ile Thr

1377 1404
CTA CGC AGC CAG CTG ACC AAC ACG CAC TGT TCA ACC TAT CCG CAC ATT GAC AAA
Leu Arg Ser Gln Leu Thr Asn Thr His Cys Ser Thr Tyr Pro His Ile Asp Lys

1431 1458
AAT TGT ACG CCT GAT GTC AAT AAA CCT TTT TCG GTA AAA GAG GTG GAT AAC AAT
Asn Cys Thr Pro Asp Val Asn Lys Pro Phe Ser Val Lys Glu Val Asp Asn Asn

1485 1512
GCC TAC AAA GAA CAG CAC AAT TTA ATC AAA GCC GTC TTT AAC AAA AAA ATG GCG
Ala Tyr Lys Glu Gln His Asn Leu Ile Lys Ala Val Phe Asn Lys Lys MET Ala

1539 1566
TTG GGC AGT ACG CAT CAT CAC ATC AAC CTG CAA GTT GGC TAT GAT AAA TTC AAT
Leu Gly Ser Thr His His His Ile Asn Leu Gln Val Gly Tyr Asp Lys Phe Asn

1593 1620
TCA AGC CTG AGC CGT GAA GAT TAT CGT TTG GCA ACC CAT CAG TCT TAT CAA AAA
Ser Ser Leu Ser Arg Glu Asp Tyr Arg Leu Ala Thr His Gln Ser Tyr Gln Lys

FIG. 5 Cont.

```
                                       1647                                  1674
CTT GAT TAC ACC CCA CCA AGT AAC CCT TTG CCA GAT AAG TTT AAG CCC ATT TTA
Leu Asp Tyr Thr Pro Pro Ser Asn Pro Leu Pro Asp Lys Phe Lys Pro Ile Leu

                                       1701                                  1728
GGT TCA AAC AAC AAA CCC ATT TGC CTT GAT GCT TAT GGT TAT GGT CAT GAC CAT
Gly Ser Asn Asn Lys Pro Ile Cys Leu Asp Ala Tyr Gly Tyr Gly His Asp His

                                       1755                                  1782
CCA CAG GCT TGT AAC GCC AAA AAC AGC ACT TAT CAA AAT TTT GCC ATC AAA AAA
Pro Gln Ala Cys Asn Ala Lys Asn Ser Thr Tyr Gln Asn Phe Ala Ile Lys Lys

                                       1809                                  1836
GGC ATA GAG CAA TAC AAC CAA AAA ACC AAT ACC GAT AAG ATT GAT TAT CAA GCC
Gly Ile Glu Gln Tyr Asn Gln Lys Thr Asn Thr Asp Lys Ile Asp Tyr Gln Ala

                                       1863                                  1890
ATC ATT GAC CAA TAT GAT AAA CAA AAC CCC AAC AGC ACC CTA AAA CCC TTT GAG
Ile Ile Asp Gln Tyr Asp Lys Gln Asn Pro Asn Ser Thr Leu Lys Pro Phe Glu

                                       1917                                  1944
AAA ATC AAA CAA AGT TTG GGG CAA GAA AAA TAC AAC AAG ATA GAC GAA CTT GGC
Lys Ile Lys Gln Ser Leu Gly Gln Glu Lys Tyr Asn Lys Ile Asp Glu Leu Gly

                                       1971                                  1998
TTT AAA GCT TAT AAA GAT TTA CGC AAC GAA TGG GCG GGT TGG ACT AAT GAC AAC
Phe Lys Ala Tyr Lys Asp Leu Arg Asn Glu Trp Ala Gly Trp Thr Asn Asp Asn

                                       2025                                  2052
AGC CAA CAA AAT GCC AAT AAA GGC ACG GAT AAT ATC TAT CAG CCA AAT CAA GCA
Ser Gln Gln Asn Ala Asn Lys Gly Thr Asp Asn Ile Tyr Gln Pro Asn Gln Ala

                                       2079                                  2106
ACT GTG GTC AAA GAT GAC AAA TGT AAA TAT AGC GAG ACC AAC AGC TAT GCT GAT
Thr Val Val Lys Asp Asp Lys Cys Lys Tyr Ser Glu Thr Asn Ser Tyr Ala Asp

                                       2133                                  2160
TGC TCA ACC ACT CGC CAC ATC AGT GGT GAT AAT TAT TTC ATC GCT TTA AAA GAC
Cys Ser Thr Thr Arg His Ile Ser Gly Asp Asn Tyr Phe Ile Ala Leu Lys Asp

                                       2187                                  2214
AAC ATG ACC ATC AAT AAA TAT GTT GAT TTG GGG CTG GGT GCT CGC TAT GAC AGA
Asn MET Thr Ile Asn Lys Tyr Val Asp Leu Gly Leu Gly Ala Arg Tyr Asp Arg
```

FIG. 5 Cont.

```
                                        2241                                          2268
ATC AAA CAC AAA TCT GAT GTG CCT TTG GTA GAC AAC AGT GCC AGC AAC CAG CTG
Ile Lys His Lys Ser Asp Val Pro Leu Val Asp Asn Ser Ala Ser Asn Gln Leu

                                        2295                                          2322
TCT TGG AAT TTT GGC GTG GTC GTC AAG CCC ACC AAT TGG CTG GAC ATC GCT TAT
Ser Trp Asn Phe Gly Val Val Val Lys Pro Thr Asn Trp Leu Asp Ile Ala Tyr

                                        2349                                          2376
AGA AGC TCG CAA GGC TTT CGC ATG CCA AGT TTT TCT GAA ATG TAT GGC GAA CGC
Arg Ser Ser Gln Gly Phe Arg MET Pro Ser Phe Ser Glu MET Tyr Gly Glu Arg

                                        2403                                          2430
TTT GGC GTA ACC ATC GGT AAA GGC ACG CAA CAT GGC TGT AAG GGT CTT TAT TAC
Phe Gly Val Thr Ile Gly Lys Gly Thr Gln His Gly Cys Lys Gly Leu Tyr Tyr

                                        2457                                          2484
ATT TGT CAG CAG ACT GTC CAT CAA ACC AAG CTA AAA CCT GAA AAA TCC TTT AAC
Ile Cys Gln Gln Thr Val His Gln Thr Lys Leu Lys Pro Glu Lys Ser Phe Asn

                                        2511                                          2538
CAA GAA ATC GGA GCG ACT TTA CAT AAC CAC TTA GGC AGT CTT GAG GTT AGT TAT
Gln Glu Ile Gly Ala Thr Leu His Asn His Leu Gly Ser Leu Glu Val Ser Tyr

                                        2565                                          2592
TTT AAA AAT CGC TAT ACC GAT TTG ATT GTT GGT AAA AGT GAA GAG ATT AGA ACC
Phe Lys Asn Arg Tyr Thr Asp Leu Ile Val Gly Lys Ser Glu Glu Ile Arg Thr

                                        2619                                          2646
CTA ACC CAA GGT GAT AAT GCA GGC AAA CAG CGT GGT AAA GGT GAT TTG GGC TTT
Leu Thr Gln Gly Asp Asn Ala Gly Lys Gln Arg Gly Lys Gly Asp Leu Gly Phe

                                        2673                                          2700
CAT AAT GGA CAA GAT GCT GAT TTG ACA GGC ATT AAC ATT CTT GGC AGA CTT GAC
His Asn Gly Gln Asp Ala Asp Leu Thr Gly Ile Asn Ile Leu Gly Arg Leu Asp

                                        2727                                          2754
CTA AAC GCT GTC AAT AGT CGC CTT CCC TAT GGA TTA TAC TCA ACA CTG GCT TAT
Leu Asn Ala Val Asn Ser Arg Leu Pro Tyr Gly Leu Tyr Ser Thr Leu Ala Tyr

                                        2781                                          2808
AAC AAA GTT GAT GTT AAA GGA AAA ACC TTA AAC CCA ACT TTG GCA GGA ACA AAC
Asn Lys Val Asp Val Lys Gly Lys Thr Leu Asn Pro Thr Leu Ala Gly Thr Asn
```

FIG. 5 Cont.

2835 2862
ATA CTG TTT GAT GCC ATC CAG CCA TCT CGT TAT GTG GTG GGG CTT GGC TAT GAT
Ile Leu Phe Asp Ala Ile Gln Pro Ser Arg Tyr Val Val Gly Leu Gly Tyr Asp

2889 2916
GCC CCA AGC CAA AAA TGG GGA GCA AAC GCC ATA TTT ACC CAT TCT GAT GCC AAA
Ala Pro Ser Gln Lys Trp Gly Ala Asn Ala Ile Phe Thr His Ser Asp Ala Lys

2943 2970
AAT CCA AGC GAG CTT TTG GCA GAT AAG AAC TTA GGT AAT GGC AAC ATT CAA ACA
Asn Pro Ser Glu Leu Leu Ala Asp Lys Asn Leu Gly Asn Gly Asn Ile Gln Thr

2997 3024
AAA CAA GCC ACC AAA GCA AAA TCC ACG CCG TGG CAA ACA CTT GAT TTG TCA GGT
Lys Gln Ala Thr Lys Ala Lys Ser Thr Pro Trp Gln Thr Leu Asp Leu Ser Gly

3051 3078
TAT GTA AAC ATA AAA GAT AAT TTT ACC TTG CGT GCT GGC GTG TAC AAT GTA TTT
Tyr Val Asn Ile Lys Asp Asn Phe Thr Leu Arg Ala Gly Val Tyr Asn Val Phe

3105 3132
AAT ACC TAT TAC ACC ACT TGG GAG GCT TTA CGC CAA ACA GCA GAA GGG GCG GTC
Asn Thr Tyr Tyr Thr Thr Trp Glu Ala Leu Arg Gln Thr Ala Glu Gly Ala Val

3159 3186
AAT CAG CAT ACA GGA CTG AGC CAA GAT AAG CAT TAT GGT CGC TAT GCC GCT CCT
Asn Gln His Thr Gly Leu Ser Gln Asp Lys His Tyr Gly Arg Tyr Ala Ala Pro

3213
GGA CGC AAT TAC CAA TTG GCA CTT GAA ATG AAG TTT TAA
Gly Arg Asn Tyr Gln Leu Ala Leu Glu MET Lys Phe .

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Figure 6. Sequence of *M. catarrhalis* 4223 *tbpB* gene

GTAAATTTGCCGTATTTTGTCTATCATAAATGCATTTATCAAATGCTCAAATAAATACGCCAAATGCACAT

TGTCAGCATGCCAAAATAGGCATCAACAGACTTTTTTAGATAATACCATCAACCCATCAGAGGATTATTTT

```
                                    27                                   54
ATG AAA CAC ATT CCT TTA ACC ACA CTG TGT GTG GCA ATC TCT GCC GTC TTA TTA
MET Lys His Ile Pro Leu Thr Thr Leu Cys Val Ala Ile Ser Ala Val Leu Leu

                                    81                                  108
ACC GCT TGT GGT GGC AGT GGT GGT TCA AAT CCA CCT GCT CCT ACG CCC ATT CCA
Thr Ala Cys Gly Gly Ser Gly Gly Ser Asn Pro Pro Ala Pro Thr Pro Ile Pro

                                   135                                  162
AAT GCT AGC GGT TCA GGT AAT ACT GGC AAC ACT GGT AAT GCT GGC GGT ACT GAT
Asn Ala Ser Gly Ser Gly Asn Thr Gly Asn Thr Gly Asn Ala Gly Gly Thr Asp

                                   189                                  216
AAT ACA GCC AAT GCA GGT AAT ACA GGC GGT ACA AAC TCT GGT ACA GGC AGT GCC
Asn Thr Ala Asn Ala Gly Asn Thr Gly Gly Thr Asn Ser Gly Thr Gly Ser Ala

                                   243                                  270
AAC ACA CCA GAG CCA AAA TAT CAA GAT GTA CCA ACT GAG AAA AAT GAA AAA GAT
Asn Thr Pro Glu Pro Lys Tyr Gln Asp Val Pro Thr Glu Lys Asn Glu Lys Asp

                                   297                                  324
AAA GTT TCA TCC ATT CAA GAA CCT GCC ATG GGT TAT GGC ATG GCT TTG AGT AAA
Lys Val Ser Ser Ile Gln Glu Pro Ala MET Gly Tyr Gly MET Ala Leu Ser Lys

                                   351                                  378
ATT AAT CTA CAC AAC CGA CAA GAC ACG CCA TTA GAT GAA AAA AAT ATC ATT ACC
Ile Asn Leu His Asn Arg Gln Asp Thr Pro Leu Asp Glu Lys Asn Ile Ile Thr

                                   405                                  432
TTA GAC GGT AAA AAA CAA GTT GCA GAA GGT AAA AAA TCG CCA TTG CCA TTT TCG
Leu Asp Gly Lys Lys Gln Val Ala Glu Gly Lys Lys Ser Pro Leu Pro Phe Ser

                                   459                                  486
TTA GAT GTA GAA AAT AAA TTG CTT GAT GGC TAT ATA GCA AAA ATG AAT GTA GCG
Leu Asp Val Glu Asn Lys Leu Leu Asp Gly Tyr Ile Ala Lys MET Asn Val Ala
```

Fig 6. (Cont.)

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513 540
GAT AAA AAT GCC ATT GGT GAC AGA ATT AAG AAA GGT AAT AAA GAA ATC TCC GAT
Asp Lys Asn Ala Ile Gly Asp Arg Ile Lys Lys Gly Asn Lys Glu Ile Ser Asp

567 594
GAA GAA CTT GCC AAA CAA ATC AAA GAA GCT GTG CGT AAA AGC CAT GAG TTT CAG
Glu Glu Leu Ala Lys Gln Ile Lys Glu Ala Val Arg Lys Ser His Glu Phe Gln

621 648
CAA GTA TTA TCA TCA CTG GAA AAC AAA ATT TTT CAT TCA AAT GAC GGA ACA ACC
Gln Val Leu Ser Ser Leu Glu Asn Lys Ile Phe His Ser Asn Asp Gly Thr Thr

675 702
AAA GCA ACC ACA CGA GAT TTA AAA TAT GTT GAT TAT GGT TAC TAC TTG GCG AAT
Lys Ala Thr Thr Arg Asp Leu Lys Tyr Val Asp Tyr Gly Tyr Tyr Leu Ala Asn

729 756
GAT GGC AAT TAT CTA ACC GTC AAA ACA GAC AAA CTT TGG AAT TTA GGC CCT GTG
Asp Gly Asn Tyr Leu Thr Val Lys Thr Asp Lys Leu Trp Asn Leu Gly Pro Val

783 810
GGT GGT GTG TTT TAT AAT GGC ACA ACG ACC GCC AAA GAG TTG CCC ACA CAA GAT
Gly Gly Val Phe Tyr Asn Gly Thr Thr Thr Ala Lys Glu Leu Pro Thr Gln Asp

837 864
GCG GTC AAA TAT AAA GGA CAT TGG GAC TTT ATG ACC GAT GTT GCC AAC AGA AGA
Ala Val Lys Tyr Lys Gly His Trp Asp Phe MET Thr Asp Val Ala Asn Arg Arg

891 918
AAC CGA TTT AGC GAA GTG AAA GAA AAC TCT CAA GCA GGC TGG TAT TAT GGA GCA
Asn Arg Phe Ser Glu Val Lys Glu Asn Ser Gln Ala Gly Trp Tyr Tyr Gly Ala

945 972
TCT TCA AAA GAT GAA TAC AAC CGC TTA TTA ACT AAA GAA GAC TCT GCC CCT GAT
Ser Ser Lys Asp Glu Tyr Asn Arg Leu Leu Thr Lys Glu Asp Ser Ala Pro Asp

999 1026
GGT CAT AGC GGT GAA TAT GGC CAT AGC AGT GAG TTT ACT GTT AAT TTT AAG GAA
Gly His Ser Gly Glu Tyr Gly His Ser Ser Glu Phe Thr Val Asn Phe Lys Glu

1053 1080
AAA AAA TTA ACA GGT AAG CTG TTT AGT AAC CTA CAA GAC CGC CAT AAG GGC AAT
Lys Lys Leu Thr Gly Lys Leu Phe Ser Asn Leu Gln Asp Arg His Lys Gly Asn

Fig. 6 (cont.)

1107 1134
GTT ACA AAA ACC GAA CGC TAT GAC ATC GAT GCC AAT ATC CAC GGC AAC CGC TTC
Val Thr Lys Thr Glu Arg Tyr Asp Ile Asp Ala Asn Ile His Gly Asn Arg Phe

1161 1188
CGT GGC AGT GCC ACC GCA AGC AAT AAA AAT GAC ACA AGC AAA CAC CCC TTT ACC
Arg Gly Ser Ala Thr Ala Ser Asn Lys Asn Asp Thr Ser Lys His Pro Phe Thr

1215 1242
AGT GAT GCC AAC AAT AGG CTA GAA GGT GGT TTT TAT GGG CCA AAA GGC GAG GAG
Ser Asp Ala Asn Asn Arg Leu Glu Gly Gly Phe Tyr Gly Pro Lys Gly Glu Glu

1269 1296
CTG GCA GGT AAA TTC TTA ACC AAT GAC AAC AAA CTC TTT GGC GTC TTT GGT GCT
Leu Ala Gly Lys Phe Leu Thr Asn Asp Asn Lys Leu Phe Gly Val Phe Gly Ala

1323 1350
AAA CGA GAG AGT AAA GCT GAG GAA AAA ACC GAA GCC ATC TTA GAT GCC TAT GCA
Lys Arg Glu Ser Lys Ala Glu Glu Lys Thr Glu Ala Ile Leu Asp Ala Tyr Ala

1377 1404
CTT GGG ACA TTT AAT ACA AGT AAC GCA ACC ACA TTC ACC CCA TTT ACC GAA AAA
Leu Gly Thr Phe Asn Thr Ser Asn Ala Thr Thr Phe Thr Pro Phe Thr Glu Lys

1431 1458
CAA CTG GAT AAC TTT GGC AAT GCC AAA AAA TTG GTC TTA GGT TCT ACC GTC ATT
Gln Leu Asp Asn Phe Gly Asn Ala Lys Lys Leu Val Leu Gly Ser Thr Val Ile

1485 1512
GAT TTG GTG CCT ACT GAT GCC ACC AAA AAT GAA TTC ACC AAA GAC AAG CCA GAG
Asp Leu Val Pro Thr Asp Ala Thr Lys Asn Glu Phe Thr Lys Asp Lys Pro Glu

1539 1566
TCT GCC ACA AAC GAA GCG GGC GAG ACT TTG ATG GTG AAT GAT GAA GTT AGC GTC
Ser Ala Thr Asn Glu Ala Gly Glu Thr Leu MET Val Asn Asp Glu Val Ser Val

1593 1620
AAA ACC TAT GGC AAA AAC TTT GAA TAC CTA AAA TTT GGT GAG CTT AGT ATC GGT
Lys Thr Tyr Gly Lys Asn Phe Glu Tyr Leu Lys Phe Gly Glu Leu Ser Ile Gly

1647 1674
GGT AGC CAT AGC GTC TTT TTA CAA GGC GAA CGC ACC GCT ACC ACA GGC GAG AAA
Gly Ser His Ser Val Phe Leu Gln Gly Glu Arg Thr Ala Thr Thr Gly Glu Lys

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Fig 6. (Cont.)

```
                                             1701                                  1728
GCC GTA CCA ACC ACA GGC ACA GCC AAA TAT TTG GGG AAC TGG GTA GGA TAC ATC
Ala Val Pro Thr Thr Gly Thr Ala Lys Tyr Leu Gly Asn Trp Val Gly Tyr Ile

                                             1755                                  1782
ACA GGA AAG GAC ACA GGA ACG GGC ACA GGA AAA AGC TTT ACC GAT GCC CAA GAT
Thr Gly Lys Asp Thr Gly Thr Gly Thr Gly Lys Ser Phe Thr Asp Ala Gln Asp

                                             1809                                  1836
GTT GCT GAT TTT GAC ATT GAT TTT GGA AAT AAA TCA GTC AGC GGT AAA CTT ATC
Val Ala Asp Phe Asp Ile Asp Phe Gly Asn Lys Ser Val Ser Gly Lys Leu Ile

                                             1863                                  1890
ACC AAA GGC CGC CAA GAC CCT GTA TTT AGC ATC ACA GGT CAA ATC GCA GGC AAT
Thr Lys Gly Arg Gln Asp Pro Val Phe Ser Ile Thr Gly Gln Ile Ala Gly Asn

                                             1917                                  1944
GGC TGG ACA GGG ACA GCC AGC ACC ACC AAA GCG GAC GCA GGA GGC TAC AAG ATA
Gly Trp Thr Gly Thr Ala Ser Thr Thr Lys Ala Asp Ala Gly Gly Tyr Lys Ile

                                             1971                                  1998
GAT TCT AGC AGT ACA GGC AAA TCC ATC GCC ATC AAA GAT GCC AAT GTT ACA GGG
Asp Ser Ser Ser Thr Gly Lys Ser Ile Ala Ile Lys Asp Ala Asn Val Thr Gly

                                             2025                                  2052
GGC TTT TAT GGT CCA AAT GCA AAC GAG ATG GGC GGG TCA TTT ACA CAC AAC GCC
Gly Phe Tyr Gly Pro Asn Ala Asn Glu MET Gly Gly Ser Phe Thr His Asn Ala

                                             2079                                  2106
GAT GAC AGC AAA GCC TCT GTG GTC TTT GGC ACA AAA AGA CAA CAA GAA GTT AAG
Asp Asp Ser Lys Ala Ser Val Val Phe Gly Thr Lys Arg Gln Gln Glu Val Lys
```

Q8 genome and subclones

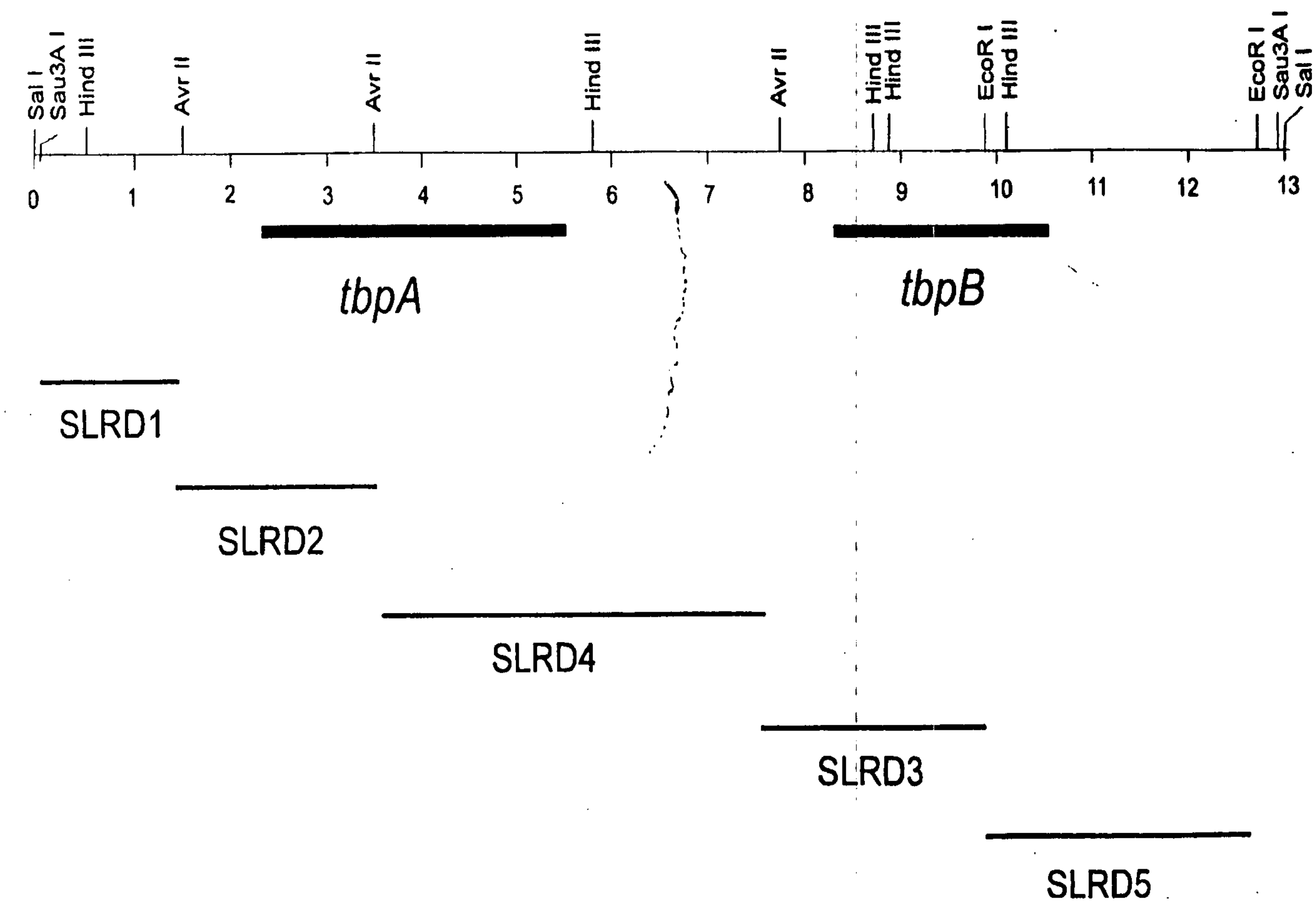

FIG. 7

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FIG.8

Chart of Q8_TBPA - Linear, length 3660

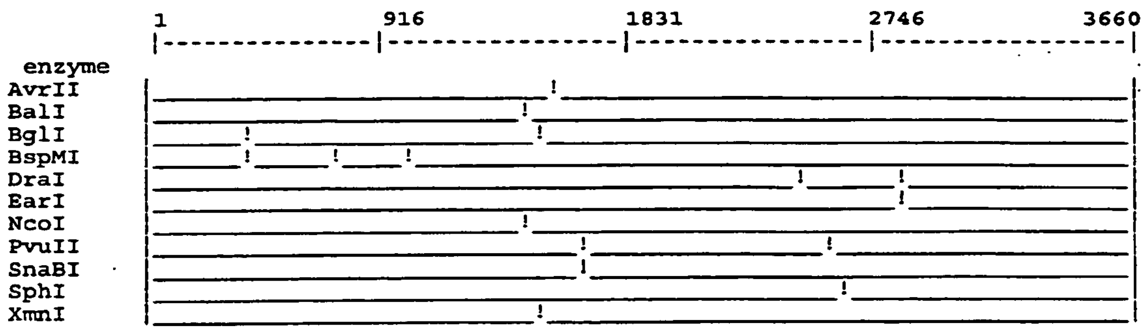

Chart of Q8_TBPB_SLRD3_SLRD5 - Linear, length 3487

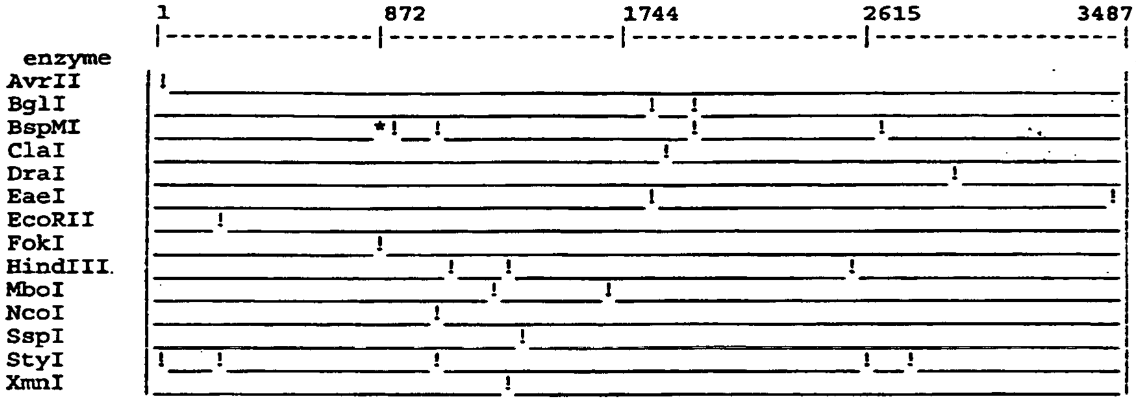

Fig. 9

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Fig. 10

Q8 tbpA gene sequence

```
AATTGATACAAAATGGTTTGTATTATCACTTGTATTTGTATTATAATTTTACTTATTTTT
         10        20        30        40        50        60

ACAAACTATACACTAAAATCAAAAATTAATCACTTTGGTTGGGTGGTTTTAGCAAGCAAA
         70        80        90       100       110       120

TGGTTATTTTGGTAAACAATTAAGTTCTTAAAAACGATACACGCTCATAAACAGATGGTT
        130       140       150       160       170       180

TTTGGCATCTTCAATTTGATGCCTGCCTTGTGATTGGTTGGGGGTGTATTGATGTATCCA
        190       200       210       220       230       240

                           MET ASN GLN SER LYS LYS SER LYS LYS SER LYS
AGTACAAAAGCCAACAGGTGGTCATTGATGAATCAATCCAAAAAATCCAAAAAATCCAAA
        250       260       270       280       290       300

GLN VAL LEU LYS LEU SER ALA LEU SER LEU GLY LEU LEU ASN ILE THR GLN VAL ALA LEU
CAAGTATTAAAACTTAGTGCCTTGTCTTTGGGTCTGCTTAACATCACGCAGGTGGCACTG
        310       320       330       340       350       360

ALA ASN THR THR ALA ASP LYS ALA GLU ALA THR ASP LYS THR ASN LEU VAL VAL VAL LEU
GCAAACACAACGGCCGATAAGGCGGAGGCAACAGATAAGACAAACCTTGTTGTTGTCTTG
        370       380       390       400       410       420

ASP GLU THR VAL VAL THR ALA LYS LYS ASN ALA ARG LYS ALA ASN GLU VAL THR GLY LEU
GATGAAACTGTTGTAACAGCGAAGAAAAACGCCCGTAAAGCCAACGAAGTTACAGGGCTT
        430       440       450       460       470       480

GLY LYS VAL VAL LYS THR ALA GLU THR ILE ASN LYS GLU GLN VAL LEU ASN ILE ARG ASP
GGTAAGGTGGTCAAAACTGCCGAGACCATCAATAAAGAACAAGTGCTAAACATTCGAGAC
        490       500       510       520       530       540

LEU THR ARG TYR ASP PRO GLY ILE ALA VAL VAL GLU GLN GLY ARG GLY ALA SER SER GLY
TTAACACGCTATGACCCTGGCATTGCTGTGGTTGAGCAAGGTCGTGGGGCAAGCTCAGGC
        550       560       570       580       590       600

TYR SER ILE ARG GLY MET ASP LYS ASN ARG VAL ALA VAL LEU VAL ASP GLY ILE ASN GLN
TATTCTATTCGTGGTATGGATAAAAATCGTGTGGCGGTATTGGTTGATGGCATCAATCAA
        610       620       630       640       650       660

ALA GLN HIS TYR ALA LEU GLN GLY PRO VAL ALA GLY LYS ASN TYR ALA ALA GLY GLY ALA
GCCCAGCACTATGCCCTACAAGGCCCTGTGGCAGGCAAAAATTATGCCGCAGGTGGGGCA
        670       680       690       700       710       720

ILE ASN GLU ILE GLU TYR GLU ASN VAL ARG SER VAL GLU ILE SER LYS GLY ALA ASN SER
ATCAACGAAATAGAATACGAAAATGTCCGCTCCGTTGAGATTAGTAAAGGTGCAAATTCA
        730       740       750       760       770       780

SER GLU TYR GLY SER GLY ALA LEU SER GLY SER VAL ALA PHE VAL THR LYS THR ALA ASP
AGTGAATACGGCTCTGGGGCATTATCTGGCTCTGTGGCATTTGTTACCAAAACCGCCGAT
        790       800       810       820       830       840

ASP ILE ILE LYS ASP GLY LYS ASP TRP GLY VAL GLN THR LYS THR ALA TYR ALA SER LYS
GACATCATCAAAGATGGTAAAGATTGGGGCGTGCAGACCAAAACCGCCTATGCCAGTAAA
        850       860       870       880       890       900

ASN ASN ALA TRP VAL ASN SER VAL ALA ALA ALA GLY LYS ALA GLY SER PHE SER GLY LEU
AATAACGCATGGGTTAATTCTGTGGCAGCAGCAGGCAAGGCAGGTTCTTTTAGCGGTCTT
        910       920       930       940       950       960

ILE ILE TYR THR ASP ARG ARG GLY GLN GLU TYR LYS ALA HIS ASP ASP ALA TYR GLN GLY
ATCATCTACACCGACCGCCGTGGTCAAGAATACAAGGCACATGATGATGCCTATCAGGGT
        970       980       990      1000      1010      1020

SER GLN SER PHE ASP ARG ALA VAL ALA THR THR ASP PRO ASN ASN PRO LYS PHE LEU ILE
AGCCAAAGTTTTGATAGAGCGGTGGCAACCACTGACCCAAATAACCCAAAATTTTTAATA
       1030      1040      1050      1060      1070      1080
```

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Fig. 10 (Cont.)

ALA ASN GLU CYS ALA ASN GLY ASN TYR GLU ALA CYS ALA ALA GLY GLY GLN THR LYS LEU
GCAAATGAATGTGCCAATGGTAATTATGAGGCGTGTGCTGCTGGCGGTCAAACCAAACTC
1090 1100 1110 1120 1130 1140

GLN ALA LYS PRO THR ASN VAL ARG ASP LYS VAL ASN VAL LYS ASP TYR THR GLY PRO ASN
CAAGCTAAGCCAACCAATGTGCGTGATAAGGTCAATGTCAAAGATTATACAGGTCCTAAC
1150 1160 1170 1180 1190 1200

ARG LEU ILE PRO ASN PRO LEU THR GLN ASP SER LYS SER LEU LEU LEU ARG PRO GLY TYR
CGCCTTATCCCAAACCCACTCACCCAAGACAGCAAATCCTTACTGCTTCGCCCAGGTTAT
1210 1220 1230 1240 1250 1260

GLN LEU ASN ASP LYS HIS TYR VAL GLY GLY VAL TYR GLU ILE THR LYS GLN ASN TYR ALA
CAGCTAAACGATAAGCACTATGTCGGTGGTGTGTATGAAATCACCAAACAAAACTACGCC
1270 1280 1290 1300 1310 1320

MET GLN ASP LYS THR VAL PRO ALA TYR LEU THR VAL HIS ASP ILE GLU LYS SER ARG LEU
ATGCAAGATAAAACCGTGCCTGCTTATCTGACGGTTCATGACATTGAAAAATCAAGGCTC
1330 1340 1350 1360 1370 1380

SER ASN HIS GLY GLN ALA ASN GLY TYR TYR GLN GLY ASN ASN LEU GLY GLU ARG ILE ARG
AGCAACCATGGCCAAGCCAATGGCTATTATCAAGGCAATAACCTTGGTGAACGCATTCGT
1390 1400 1410 1420 1430 1440

ASP ALA ILE GLY ALA ASN SER GLY TYR GLY ILE ASN TYR ALA HIS GLY VAL PHE TYR ASP
GATGCCATTGGGGCAAATTCAGGTTATGGCATCAACTATGCTCATGGCGTATTTTATGAC
1450 1460 1470 1480 1490 1500

GLU LYS HIS GLN LYS ASP ARG LEU GLY LEU GLU TYR VAL TYR ASP SER LYS GLY GLU ASN
GAAAAACACCAAAAAGACCGCCTAGGGCTTGAATATGTTTATGACAGCAAAGGTGAAAAT
1510 1520 1530 1540 1550 1560

LYS TRP PHE ASP ASP VAL ARG VAL SER TYR ASP LYS GLN ASP ILE THR LEU ARG SER GLN
AAATGGTTTGATGATGTGCGTGTGTCTTATGACAAGCAAGACATTACGCTACGTAGCCAG
1570 1580 1590 1600 1610 1620

LEU THR ASN THR HIS CYS SER THR TYR PRO HIS ILE ASP LYS ASN CYS THR PRO ASP VAL
CTGACCAACACGCACTGTTCAACCTATCCGCACATTGACAAAAATTGTACGCCTGATGTC
1630 1640 1650 1660 1670 1680

ASN LYS PRO PHE SER VAL LYS GLU VAL ASP ASN ASN ALA TYR LYS GLU GLN HIS ASN LEU
AATAAACCTTTTTCGGTAAAAGAGGTGGATAACAATGCCTACAAAGAACAGCACAATTTA
1690 1700 1710 1720 1730 1740

ILE LYS ALA VAL PHE ASN LYS LYS MET ALA LEU GLY ASN THR HIS HIS HIS ILE ASN LEU
ATCAAAGCCGTCTTTAACAAAAAAATGGCATTGGGCAATACGCATCATCACATCAATCTG
1750 1760 1770 1780 1790 1800

GLN VAL GLY TYR ASP LYS PHE ASN SER SER LEU SER ARG GLU ASP TYR ARG LEU ALA THR
CAAGTTGGCTATGATAAATTCAATTCAAGCCTTAGCCGTGAAGATTATCGTTTGGCAACC
1810 1820 1830 1840 1850 1860

HIS GLN SER TYR GLN LYS LEU ASP TYR THR PRO PRO SER ASN PRO LEU PRO ASP LYS PHE
CATCAATCTTATCAAAAACTTGATTACACCCCACCAAGTAACCCTTTGCCAGATAAGTTT
1870 1880 1890 1900 1910 1920

LYS PRO ILE LEU GLY SER ASN ASN ARG PRO ILE CYS LEU ASP ALA TYR GLY TYR GLY HIS
AAGCCCATTTTAGGTTCAAACAACAGACCCATTTGCCTTGATGCTTATGGTTATGGTCAT
1930 1940 1950 1960 1970 1980

ASP HIS PRO GLN ALA CYS ASN ALA LYS ASN SER THR TYR GLN ASN PHE ALA ILE LYS LYS
GACCATCCACAGGCTTGTAACGCCAAAAACAGCACTTATCAAAACTTTGCCATCAAAAAA
1990 2000 2010 2020 2030 2040

GLY ILE GLU GLN TYR ASN GLN THR ASN THR ASP LYS ILE ASP TYR GLN ALA VAL ILE ASP
GGCATAGAGCAATACAACCAAACCAATACCGATAAGATTGATTATCAAGCCGTCATTGAC
2050 2060 2070 2080 2090 2100

GLN TYR ASP LYS GLN ASN PRO ASN SER THR LEU LYS PRO PHE GLU LYS ILE LYS GLN SER
CAATATGATAAACAAAACCCCAACAGCACCCTAAAACCCTTTGAGAAAATCAAACAAAGT
2110 2120 2130 2140 2150 2160

Fig. 10 (Cont.)

LEU GLY GLN GLU LYS TYR ASP GLU ILE ASP ARG LEU GLY PHE ASN ALA TYR LYS ASP LEU
T T G G G G C A A G A A A A A T A C G A C G A G A T A G A C A G A C T G G G C T T T A A T G C T T A T A A A G A T T T A
2170 2180 2190 2200 2210 2220

ARG ASN GLU TRP ALA GLY TRP THR ASN ASP ASN SER GLN GLN ASN ALA ASN LYS GLY THR
C G C A A C G A A T G G G C G G G T T G G A C T A A T G A C A A C A G C C A A C A A A A C G C C A A T A A A G G C A C G
2230 2240 2250 2260 2270 2280

ASP ASN ILE TYR GLN PRO ASN GLN ALA THR VAL VAL LYS ASP ASP LYS CYS LYS TYR SER
G A T A A T A T C T A T C A G C C A A A T C A A G C A A C T G T G G T C A A A G A T G A C A A A T G T A A A T A T A G C
2290 2300 2310 2320 2330 2340

GLU THR ASN SER TYR ALA ASP CYS SER THR THR ARG HIS ILE SER GLY ASP ASN TYR PHE
G A G A C C A A C A G C T A T G C T G A T T G C T C A A C C A C T C G C C A C A T C A G C G G T G A T A A T T A T T T C
2350 2360 2370 2380 2390 2400

ILE ALA LEU LYS ASP ASN MET THR ILE ASN LYS TYR VAL ASP LEU GLY LEU GLY ALA ARG
A T C G C T T T A A A A G A C A A C A T G A C C A T C A A T A A A T A T G T T G A T T T G G G G C T G G G T G C T C G C
2410 2420 2430 2440 2450 2460

TYR ASP ARG ILE LYS HIS LYS SER ASP VAL PRO LEU VAL ASP ASN SER ALA SER ASN GLN
T A T G A C A G A A T C A A A C A C A A A T C T G A T G T G C C T T T G G T A G A C A A C A G T G C C A G C A A C C A G
2470 2480 2490 2500 2510 2520

LEU SER TRP ASN PHE GLY VAL VAL VAL LYS PRO THR ASN TRP LEU ASP ILE ALA TYR ARG
C T G T C T T G G A A T T T T G G C G T G G T C G T C A A G C C C A C C A A T T G G C T G G A C A T C G C T T A T A G A
2530 2540 2550 2560 2570 2580

SER SER GLN GLY PHE ARG MET PRO SER PHE SER GLU MET TYR GLY GLU ARG PHE GLY VAL
A G C T C G C A A G G C T T T C G C A T G C C A A G T T T T T C T G A A A T G T A T G G C G A A C G C T T T G G C G T A
2590 2600 2610 2620 2630 2640

THR ILE GLY LYS GLY THR GLN HIS GLY CYS LYS GLY LEU TYR TYR ILE CYS GLN GLN THR
A C C A T C G G T A A A G G C A C G C A A C A T G G C T G T A A G G G T C T T T A T T A C A T T T G T C A G C A G A C T
2650 2660 2670 2680 2690 2700

VAL HIS GLN THR LYS LEU LYS PRO GLU LYS SER PHE ASN GLN GLU ILE GLY ALA THR LEU
G T C C A T C A A A C C A A G C T A A A A C C T G A A A A A T C C T T T A A C C A A G A A A T C G G A G C G A C T T T A
2710 2720 2730 2740 2750 2760

HIS ASN HIS LEU GLY SER LEU GLU VAL SER TYR PHE LYS ASN ARG TYR THR ASP LEU ILE
C A T A A C C A C T T A G G C A G T C T T G A G G T T A G T T A T T T T A A A A A T C G C T A T A C C G A T T T G A T T
2770 2780 2790 2800 2810 2820

VAL GLY LYS SER GLU GLU ILE ARG THR LEU THR GLN GLY ASP ASN ALA GLY LYS GLN ARG
G T T G G T A A A A G T G A A G A G A T T A G A A C C C T A A C C C A A G G T G A T A A T G C A G G C A A A C A G C G T
2830 2840 2850 2860 2870 2880

GLY LYS GLY ASP LEU GLY PHE HIS ASN GLY GLN ASP ALA ASP LEU THR GLY ILE ASN ILE
G G T A A A G G T G A T T T G G G C T T T C A T A A T G G G C A A G A T G C T G A T T T G A C A G G C A T T A A C A T T
2890 2900 2910 2920 2930 2940

LEU GLY ARG LEU ASP LEU ASN ALA VAL ASN SER ARG LEU PRO TYR GLY LEU TYR SER THR
C T T G G C A G A C T T G A C C T A A A C G C T G T C A A T A G T C G C C T T C C C T A T G G A T T A T A C T C A A C A
2950 2960 2970 2980 2990 3000

LEU ALA TYR ASN LYS VAL ASP VAL LYS GLY LYS THR LEU ASN PRO THR LEU ALA GLY THR
C T G G C T T A T A A C A A A G T T G A T G T T A A A G G A A A A A C C T T A A A C C C A A C T T T G G C A G G A A C A
3010 3020 3030 3040 3050 3060

ASN ILE LEU PHE ASP ALA ILE GLN PRO SER ARG TYR VAL VAL GLY LEU GLY TYR ASP ALA
A A C A T A C T G T T T G A T G C C A T T C A G C C A T C T C G T T A T G T G G T G G G G C T T G G C T A T G A T G C C
3070 3080 3090 3100 3110 3120

PRO SER GLN LYS TRP GLY ALA ASN ALA ILE PHE THR HIS SER ASP ALA LYS ASN PRO SER
C C A A G C C A A A A A T G G G G A G C A A A C G C C A T A T T T A C C C A T T C T G A T G C C A A A A A T C C A A G C
3130 3140 3150 3160 3170 3180

GLU LEU LEU ALA ASP LYS ASN LEU GLY ASN GLY ASN ILE GLN THR LYS GLN ALA THR LYS
G A G C T T T T G G C A G A T A A G A A C T T A G G T A A T G G C A A C A T T C A A A C A A A A C A A G C C A C C A A A
3190 3200 3210 3220 3230 3240

Fig. 10 (cont.)

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ALA LYS SER THR PRO TRP GLN THR LEU ASP LEU SER GLY TYR VAL ASN ILE LYS ASP ASN
GCAAAATCCACGCCGTGGCAAACACTTGATTTGTCAGGTTATGTAAACATAAAAGATAAT
         3250      3260      3270      3280      3290      3300

PHE THR LEU ARG ALA GLY VAL TYR ASN VAL PHE ASN THR TYR TYR THR THR TRP GLU ALA
TTTACCTTGCGTGCTGGCGTGTACAATGTATTTAATACCTATTACACCACTTGGGAGGCT
         3310      3320      3330      3340      3350      3360

LEU ARG GLN THR ALA GLU GLY ALA VAL ASN GLN HIS THR GLY LEU SER GLN ASP LYS HIS
TTACGCCAAACAGCAGAAGGGGCGGTCAATCAGCATACAGGACTGAGCCAAGATAAGCAT
         3370      3380      3390      3400      3410      3420

TYR GLY ARG TYR ALA ALA PRO GLY ARG ASN TYR GLN LEU ALA LEU GLU MET LYS PHE ***
TATGGTCGCTATGCCGCTCCTGGACGCAATTACCAATTGGCACTTGAAATGAAGTTTTAA
         3430      3440      3450      3460      3470      3480

CCAGTGGCTTTGATGTGATCATGCCAAATCCCAATCAACCAATGAATAAAGCCCCCATCT
         3490      3500      3510      3520      3530      3540

ACCATGAGGGCTTTATTTTATCATCGCTGAGTATGCTCTTAGCGGTCATCACTCAGATTA
         3550      3560      3570      3580      3590      3600

GTCATTAATTTATTAGCGATTAATTTATTAGTAATCACGCTGCTCTTTGATGATTTTAAG
         3610      3620      3630      3640      3650      3660
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<u>Fig. 11</u>

Q8 tbpB Sequence

~~SLRD3 and SLRD5 sequences of Q8 tbpB~~
~~IS THE SEQUENCE BEING TRANSLATED~~

```
CCTAGGGCTGACAGTAACAACACTTTATACAGCACATCATTGATTTATTACCCAAATGCC
        10        20        30        40        50        60

ACACGCTATTATCTTTTGGGGGCAGACTTTTATGATGAAAAAGTGCCACAAGACCCATCT
        70        80        90       100       110       120

GACAGCTATGAGCGTCGTGGCATACGCACAGCTTGGGGGCAAGAATGGGCGGGCGGTCTT
       130       140       150       160       170       180

TCAAGCCGTGCCCAAATCAGCATCAACAAACGCCATTACCAAGGAGCAAACCTAACCAGC
       190       200       210       220       230       240

GGTGGACAAATTCGCCAGGATAAACAGATGCAAGCGTCTTTATCGCTTTGGCACAGAGAC
       250       260       270       280       290       300

ATTCACAAATGGGGCATCACGCCACGGCTGACCATCAGCACAAACATCAATAAAAGCAAT
       310       320       330       340       350       360

GACATCAAGGCAAATTATCACAAAAATCAAATGTTTGTTGAGTTTAGTCGCATTTTTTGA
       370       380       390       400       410       420

TGGGATAAGCATGCCCTACTTTTGTTTTTTGTAAAAAAATGTACCATCATAGACAATATC
       430       440       450       460       470       480

AAGAAAAAATCAAGAAAAAAGATTACAAATTTAATGATAATTGTTATTGTTTATGTTATT
       490       500       510       520       530       540

ATTTATCAATGTAAATTTGCCGTATTTTGTCCATCATAAACGCATTTATCAAATGCTCAA
       550       560       570       580       590       600

ATAAATACGCCAAATGCACATTGTCAACATGCCAAAATAGGCATTAACAGACTTTTTTAG
       610       620       630       640       650       660

                                  MET LYS HIS ILE PRO LEU THR THR LEU C
ATAATACCATCAACCCATCAGAGGATTATTTTATGAAACACATTCCTTTAACCACACTGT
       670       680       690       700       710       720

YS VAL ALA ILE SER ALA VAL LEU LEU THR ALA CYS GLY GLY SER SER GLY GLY PHE ASN P
GTGTGGCAATCTCTGCCGTCTTATTAACCGCTTGTGGTGGTAGCAGTGGTGGTTTCAATC
       730       740       750       760       770       780

RO PRO ALA SER THR PRO ILE PRO ASN ALA GLY ASN SER GLY ASN ALA GLY ASN ALA GLY A
CACCTGCCTCTACGCCCATCCCAAATGCAGGTAATTCAGGTAATGCTGGCAATGCTGGCA
       790       800       810       820       830       840

SN ALA GLY GLY THR GLY GLY ALA ASN SER GLY ALA GLY ASN ALA GLY GLY THR GLY GLY A
ATGCTGGCGGTACTGGCGGTGCAAACTCTGGTGCAGGTAATGCTGGCGGTACTGGCGGTG
       850       860       870       880       890       900

LA ASN SER GLY ALA GLY SER ALA SER THR PRO GLU PRO LYS TYR LYS ASP VAL PRO THR A
CAAACTCTGGTGCAGGCAGTGCCAGCACACCAGAACCAAAATATAAAGATGTGCCAACCG
       910       920       930       940       950       960

SP GLU ASN LYS LYS ALA GLU VAL SER GLY ILE GLN GLU PRO ALA MET GLY TYR GLY VAL G
ATGAAAATAAAAAAGCTGAAGTTTCAGGCATTCAAGAACCTGCCATGGGTTATGGCGTGG
       970       980       990      1000      1010      1020
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Fig. 11 (Cont.)

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LU  LEU LYS LEU ARG ASN TRP ILE PRO GLN GLU GLN GLU GLU HIS ALA LYS ILE ASN THR A
AATTAAAGCTTCGTAACTGGATACCACAAGAACAGGAAGAACATGCCAAAATCAATACAA
      1030      1040      1050      1060      1070      1080

SN  ASP VAL VAL LYS LEU GLU GLY ASP LEU LYS HIS ASN PRO PHE ASP ASN SER ILE TRP G
ATGATGTTGTAAAACTTGAAGGTGACTTGAAGCATAATCCATTTGACAACTCTATTTGGC
      1090      1100      1110      1120      1130      1140

LN  ASN ILE LYS ASN SER LYS GLU VAL GLN THR VAL TYR ASN GLN GLU LYS GLN ASN ILE G
AAAACATCAAAAATAGCAAAGAAGTACAAACTGTTTACAACCAAGAGAAGCAAAACATTG
      1150      1160      1170      1180      1190      1200

LU  ASP GLN ILE LYS ARG GLU ASN LYS GLN ARG PRO ASP LYS LYS LEU ASP ASP VAL ALA L
AAGATCAAATCAAAAGAGAAAATAAACAACGCCCTGACAAAAAACTTGATGACGTGGCAC
      1210      1220      1230      1240      1250      1260

EU  GLN ALA TYR ILE GLU LYS VAL LEU ASP ASP ARG LEU THR GLU LEU ALA LYS PRO ILE T
TACAAGCTTATATTGAAAAAGTTCTTGATGACCGTCTAACAGAACTTGCTAAACCCATTT
      1270      1280      1290      1300      1310      1320

YR  GLU LYS ASN ILE ASN TYR SER HIS ASP LYS GLN ASN LYS ALA ARG THR ARG ASP LEU L
ATGAAAAAAATATTAATTATTCACATGATAAGCAGAATAAAGCACGCACTCGTGATTTGA
      1330      1340      1350      1360      1370      1380

YS  TYR VAL ARG SER GLY TYR ILE TYR ARG SER GLY TYR SER ASN ILE ILE PRO LYS LYS I
AGTATGTGCGTTCTGGTTATATTTATCGCTCAGGTTATTCTAATATCATTCCAAAGAAAA
      1390      1400      1410      1420      1430      1440

LE  ALA LYS THR GLY PHE ASP GLY ALA LEU PHE TYR GLN GLY THR GLN THR ALA LYS GLN L
TAGCTAAAACTGGTTTTGATGGTGCTTTATTTTATCAAGGTACACAAACTGCTAAACAAT
      1450      1460      1470      1480      1490      1500

EU  PRO VAL SER GLN VAL LYS TYR LYS GLY THR TRP ASP PHE MET THR ASP ALA LYS LYS G
TGCCTGTATCTCAAGTTAAGTATAAAGGCACTTGGGATTTTATGACCGATGCCAAAAAAG
      1510      1520      1530      1540      1550      1560

LY  GLN SER PHE SER SER PHE GLY THR SER GLN ARG LEU ALA GLY ASP ARG TYR SER ALA M
GACAATCATTTAGCAGTTTTGGTACATCGCAACGTCTTGCTGGTGATCGTTATAGTGCAA
      1570      1580      1590      1600      1610      1620

ET  SER TYR HIS GLU TYR PRO SER LEU LEU THR ASP GLU LYS ASN LYS PRO ASP ASN TYR A
TGTCTTACCATGAATACCCATCTTTATTAACTGATGAGAAAAACAAACCAGATAATTATA
      1630      1640      1650      1660      1670      1680

SN  GLY GLU TYR GLY HIS SER SER GLU PHE THR VAL ASP PHE SER LYS LYS SER LEU LYS G
ACGGTGAATATGGTCATAGCAGTGAGTTTACGGTAGATTTTAGTAAAAAGAGCCTAAAAG
      1690      1700      1710      1720      1730      1740

LY  GLU LEU SER SER ASN ILE GLN ASP GLY HIS LYS GLY SER VAL ASN LYS THR LYS ARG T
GTGAGCTGTCTAGTAACATACAAGACGGCCATAAGGGCAGTGTTAATAAAACCAAACGCT
      1750      1760      1770      1780      1790      1800

YR  ASP ILE ASP ALA ASN ILE TYR GLY ASN ARG PHE ARG GLY SER ALA THR ALA SER ASP T
ATGACATCGATGCCAATATCTACGGCAACCGCTTCCGTGGCAGTGCCACCGCAAGCGATA
      1810      1820      1830      1840      1850      1860

HR  THR GLU ALA SER LYS SER LYS HIS PRO PHE THR SER ASP ALA LYS ASN SER LEU GLU G
CAACAGAAGCAAGCAAAAGCAAACACCCCTTTACCAGCGATGCCAAAAATAGCCTAGAAG
      1870      1880      1890      1900      1910      1920

LY  GLY PHE TYR GLY PRO ASN ALA GLU GLU LEU ALA GLY LYS PHE LEU THR ASN ASP ASN L
GCGGTTTTTATGGACCAAACGCCGAGGAGCTGGCAGGTAAATTCCTAACCAATGACAACA
      1930      1940      1950      1960      1970      1980

YS  LEU PHE GLY VAL PHE GLY ALA LYS ARG GLU SER GLU ALA LYS GLU LYS THR GLU ALA I
AACTCTTTGGCGTCTTTGGTGCTAAACGAGAGAGTGAAGCTAAGGAAAAAACCGAAGCCA
      1990      2000      2010      2020      2030      2040

LE  LEU ASP ALA TYR ALA LEU GLY THR PHE ASN LYS PRO GLY THR THR ASN PRO ALA PHE T
TCTTAGATGCCTATGCACTTGGGACATTTAATAAACCTGGTACGACCAATCCCGCCTTTA
      2050      2060      2070      2080      2090      2100
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Fig. 11 (Cont.)

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HR  ALA ASN SER LYS LYS GLU LEU ASP ASN PHE GLY ASN ALA LYS LYS LEU VAL LEU GLY S
CCGCTAACAGCAAAAAAGAACTGGATAACTTTGGCAATGCCAAAAAGTTGGTCTTGGGTT
         2110      2120      2130      2140      2150      2160

ER  THR VAL ILE ASP LEU VAL PRO THR GLY ALA THR LYS ASP VAL ASN GLU PHE LYS GLU L
CTACCGTCATTGATTTGGTGCCTACCGGTGCCACCAAAGATGTCAATGAATTCAAAGAAA
         2170      2180      2190      2200      2210      2220

YS  PRO LYS SER ALA THR ASN LYS ALA GLY GLU THR LEU MET VAL ASN ASP GLU VAL ILE V
AGCCAAAGTCTGCCACAAACAAAGCGGGCGAGACTTTGATGGTGAATGATGAAGTTATCG
         2230      2240      2250      2260      2270      2280

AL  LYS THR TYR GLY TYR GLY ARG ASN PHE GLU TYR LEU LYS PHE GLY GLU LEU SER ILE G
TCAAAACCTATGGCTATGGCAGAAACTTTGAATACCTAAAATTTGGTGAGCTTAGTATCG
         2290      2300      2310      2320      2330      2340

LY  GLY SER HIS SER VAL PHE LEU GLN GLY GLU ARG THR ALA GLU LYS ALA VAL PRO THR G
GTGGTAGCCATAGCGTCTTTTTACAAGGCGAACGCACCGCTGAGAAAGCCGTACCAACCG
         2350      2360      2370      2380      2390      2400

LU  GLY THR ALA LYS TYR LEU GLY ASN TRP VAL GLY TYR ILE THR GLY LYS ASP THR GLY T
AAGGCACAGCCAAATATCTGGGGAACTGGGTAGGATACATCACAGGAAAGGACACAGGAA
         2410      2420      2430      2440      2450      2460

HR  SER THR GLY LYS SER PHE ASN GLU ALA GLN ASP ILE ALA ASP PHE ASP ILE ASP PHE G
CGAGCACAGGAAAAAGCTTTAATGAGGCCCAAGATATTGCTGATTTTGACATTGACTTTG
         2470      2480      2490      2500      2510      2520

LU  ARG LYS SER VAL LYS GLY LYS LEU THR THR GLN GLY ARG GLN ASP PRO VAL PHE ASN I
AGAGAAAATCAGTTAAAGGCAAACTGACCACCCAAGGCCGCCAAGACCCTGTATTTAACA
         2530      2540      2550      2560      2570      2580

LE  THR GLY GLN ILE ALA GLY ASN GLY TRP THR GLY THR ALA SER THR ALA LYS ALA ASN V
TCACAGGTCAAATCGCAGGTAATGGCTGGACAGGCACAGCCAGCACCGCCAAAGCGAACG
         2590      2600      2610      2620      2630      2640

AL  GLY GLY TYR LYS ILE ASP SER SER SER THR GLY LYS SER ILE VAL ILE GLU ASN ALA L
TAGGGGGCTACAAGATAGATTCTAGCAGTACAGGCAAATCCATCGTCATCGAAAATGCCA
         2650      2660      2670      2680      2690      2700

YS  VAL THR GLY GLY PHE TYR GLY PRO ASN ALA ASN GLU MET GLY GLY SER PHE THR HIS A
AGGTTACAGGTGGCTTTTATGGTCCAAATGCAAACGAGATGGGCGGGTCATTTACACACG
         2710      2720      2730      2740      2750      2760

SP  THR ASP ASP SER LYS ALA SER VAL VAL PHE GLY THR LYS ARG GLN GLU GLU VAL LYS *
ATACCGATGACAGTAAAGCCTCTGTGGTCTTTGGCACAAAAAGACAAGAAGAAGTTAAGT
         2770      2780      2790      2800      2810      2820

**
AGTAATTTAAACACAATGCTTGGTTCGGCTGATGGGATTGACGCTTAATCAAACATGAAT
         2830      2840      2850      2860      2870      2880

GATTAAGATGATAAACCCAAGCCATGCCAATGATTGATAGCAACGATGGCAGATGATGAG
         2890      2900      2910      2920      2930      2940

TTTTCATTATCTGCCATTATTATTGCTTAATTATTGCTTGTCATTTGGTGGTGTTATCAC
         2950      2960      2970      2980      2990      3000

ATTAATCATTAAAATTAACATAATAAATGATTAAATGATATTTAATGAAAGTCAGGGTTA
         3010      3020      3030      3040      3050      3060

TTTTGGTCATGGTTTTTCATGATTATTTAACTTATAATGCGTTATGGTTAGCAAAAAGCT
         3070      3080      3090      3100      3110      3120

AAGTCTGTCAATGAAGCTATGGTGAGTGATTGTGCAAAAGATGGTCAAAAAAATCGGTAT
         3130      3140      3150      3160      3170      3180
```

Fig. 11 (Cont.)

```
G G T G C T G T C A G G C G T G G T G A T G G T T C T G T T A A T G A T A A T A A C A A C G C C A A G C C A T G C T A C
                  3190                3200                3210                3220                3230                3240

T G C C A A G T T G T T G C C G A C C T C T C A A G A A A A T C C A A C C A A A A C T A T G G T A G A T A G C T T T G G
                  3250                3260                3270                3280                3290                3300

T C G T G A A A C G C C A C G A G G G G C A G T T C A G G G G C T A T T G C G T G C A A T T G C A G C A G A A G A C T A
                  3310                3320                3330                3340                3350                3360

T G A G C T G G C T G C C A A C T A T T T G G A C G G C C G T T A T T T G G C A A A A A C C C A A A C G C C C A A T C G
                  3370                3380                3390                3400                3410                3420

T G A G A T T G T T G A G C A
                  3430
```

Fig. 12

Tbp1 alignment

```
        10          20          30        40          50         60          70         80         90         100
MNQSKQNNKSKKSKQVLKLSALSLGLLNI--TQVALANTTADKAEA-TDKTNLVVVLDETVVTAKKNA-RKANEVTGLGKVVKTAETINKEQVLNIRDLTRYDP      4223
....---.......................--...............-.....................-...................................      Q8
.Q.QHLFR--------.NILC..---------MT..PVY----..NVQAEQAQEKQ..TIQ.K...QKT.RD........L..SSD.LS...............      B16B6
.Q.QHLFR--------.NILC..---------MT..PAY----..NVQAGQAQEKQ..TIQ.K...QKT.RD........L...D.LS.....D.........      M982
.Q.QHLFR--------.NILC..---------MT..PAY----..NVQAGQAQEKQ..TIQ.K...QKT.RD........L...D.LS.....D.........      FA19
.TKKPYFR-------LSIISC.LI.CYVKAE..SIKDTKE.ISS.VD.QS.E-DSE.ETIS...E.IRD..D........II..S.S.SR..............      Eagan

       110        120        130        140        150        160        170        180        190       200
GIAVVEQGRGASSGYSIRGMDKNRVAVLVDGINQAQHYALQGPVAGKNYA-AGGAINEIEYENVRSVEISKGANSSEYGSGALSGSVAFVTKTADDIIKDG      4223
..................................................-.................................................      Q8
.......................SLT...VS.I.S.TA.AALG.TRT.GSS...........KA......S......N...A.....Q....A...GE.      B16B6
.......................SLT...LA.I.S.TA.AALG.TRT.GSS...........KA......S..V.Q.....A.....Q......V.GE.      M982
.......................SLT...LA.I.S.TA.AALG.TRT.GSS...........KA......S..V.Q.....A.....Q......V.GE.      FA19
..S.................R....L....LP.T.S.VV.S.LVARSGYSGT...........KA......GS.....N...A...T.QS.S.A..LEGD      Eagan

       210        220        230        240        250        260               270        280        290        300
KDWGVQTKTAYASKNNAWVNSVAAAGKAGSFSGLIIYTDRRGQEYKAHDDAYQGSQSFDRAVATTD-----PNNRTFLIANECANGNYEACAAGGQTKLQAKPTN      4223
..................................................................-----..PK..............................      Q8
.Q..I.S....SG.DH.LTQ.L.L..RS.GAEA.L...K...R.IH..K..GK.V...N.L.LDE.KKEGGSQY.Y.IVEE..H..-.A..KNKL--.ED.SVKD      B16B6
RQ..I.S....SG..RGLTQ.I.L..RI.GAEA.L.H.G..AG.IR..E..GR.V...N.L.PVE.----SSEYAY.IVED..EGK...T.KSKP--.KDVVGKD      M982
RQ..I.S....SG..RGLTQ.I.L..RI.GAEA.L.R.G.HAG.IR..EA.GR.V...N.LAPVD.----GSKYAY.IVEE..K..GH.K.K.NP--.KDVVGED      FA19
.S..I...N..S...KGFTH.L.V...Q.G.E..A...Q.NSI.TQV.K..LK.V..Y..LI...-----KSSGY.V.QG..P..DDK-...----.PP.TLST      Eagan

       310        320        330        340        350        360        370        380         390       400
VRDKVNVKDYTGPNRLIPNPLTQDSKSLLLRPGYQLNDK-HYVGGVYEITKQNYAMQDKTVPAYLTVHDIEKSRLSNHAQA--NGYYQGNNLGERIRDTIGPD      4223
.......................................-.......................................G.--...............A..AN      Q8
E.KT.STQ....S...LA...EYG.Q.W.F...WH.DNR-....A.L.R.Q.TFDTR.M.....F.SE.YVPGS.KGL------.K.S.D.KA..LFVQGEGS      B16B6
E.QT.STR.......FLAD..SYE.R.W.F...FRFENKR..I..IL.H.Q.TFDTR.M....F..KAVFDANSKQAGSLPG-..K.A..HKYGGLFTNGENG      M982
K.QT.STR.......FLAD..SYE.R.W.F...FRFENKR..I..IL.R.Q.TFDTR.M....F..KAVFDANQKQAGSLRG-..K.A..HKYGGLFTSGENN      FA19
QSET.S.S....A..IK...MKYE.Q.WF..G..HFSEQ-..I..IF.F.Q.KFDIR.M.F...SPTERRDDSSRSFYPMQDH.A..HIE-----------      Eagan

           410        420        430        440        450        460        470        480        490       500
----SGYGINYAHGVFYDEKHQKDRLGLEYVYDSKGENKWFDDVRVSYDKQDITLRSQLTNTHCSTYPHIDKNCTPDVNKPFSVKEVDNNAYKEQHNLIKAVFN      4223
----................................................................................................      Q8
TLQGI..----T......R.T.N.Y.V....HNADKDT.A.YA.L...R.G.D.DNR.QQ....HDGS-....R..G...Y.FYKS.RMI.E.SR..FQ...K      B16B6
----ALV.AE.GT......T.T.S.Y......TNADKDT.A.YA.L...R.G.G.DNHFQQ....ADGS-..Y.R.SAD....YYKS.RVI.G.S.R.LQ.A.K      M982
----APV.AE.GT......T.T.S.Y......TNADKDT.A.YA.L...R.G.G.DNHFQQ....ADGS-..Y.R.SAD....YYKS.RVI.G.S.K.LQ.A.K      FA19
----D.R.VK..S.LYF..H.R.Q.V.I...I.EN.NKAGII.KAVL.ANQ.N.I.D.YMRH....L..NPS...R.TLD..Y.YYRS.R.V...K..MLQLNLE      Eagan

       510        520        530        540        550        560        570        580        590       600
KKMALGSTHHHINLQVGYDKFNSSLSREDYRLATHQSYQKLDYTPPSNPLPDKF-KPILGSNNKPICLDAYGYGHDHPQACNAKNSTYQNFAIKKGIEQYN      4223
......N...............................................-........R....................................      Q8
.AFDTAKIR.NLSINL...R.K.Q..HS..Y.QNAVQAYD.I-...KP.F.NGS-.------DN.YRVSIGK-----------------------------      B16B6
.SFDTAKIR.NLSVNL.F.R.D.N.RHQ..YYQHANRAYSSK-...KTAN.NGD-.------S..YWVSIG.-----------------------------      M982
.SFDTAKIR.NLSVNL...R.G.N.RHQ..YYQSANRAYS.K-...Q.NGKKTS---PN.REKN.YWVSIGR-----------------------------      FA19
..IQQNWLT.Q.VFNL.F.D.T.A.QHK..-.TRRVIATA-.SI.RK---.GETG..RN.LQSQ.YLYPKPEP----------------------------      Eagan
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Fig. 12 (Cont.)

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         610       620       630       640       650       660       670       680       690       700
QKTNTDKIDYQAIIDQYDKQNPNSTLKPFEKIKQSLGQEKYNKIDELGFKAYKDLRNEWAGWTNDNSQQNANKGTDNIYQPNQA-TVVKDDKCKYSETNS-Y  4223
.-..........V..........................DE..R...N...................................-...........-.  Q8
------------------------------------------------------------------------------------T..NTSPI.RFGN-.T-.  B16B6
------------------------------------------------------------------------------------GN..TGQI.LFGN-.T-.  M982
------------------------------------------------------------------------------------GN..TRQI.LFGN-.T-.  FA19
------------------------------------------------------------------------------------YFAGQDH-.N.QGSS.N.  Eagan

         710       720       730       740       750       760       770       780       790       800
ADCSTTRHISGDNYFIALKDNMTINKYVDLGLGARYDRIKHKSDVPLVDNSASNQLSWNFGVVVKPTNWLDIAYRSSQGFRMPSFSEMYGERFGVTIGKG  4223
....................................................................................................  Q8
T..-.P.N.G.NG.YA.VQ..VRLGRWA.V.A.I...YRSTH.EDKS.STGTHRN...A...L..FT.M.LT..A.T...L...A....W.A.ESLKTL  B16B6
T..-.P.S.N.KS.YA.VR..VRLGRWA.V.A.L...YRSTH..DGS.STGTHRT...A.I.L..AD...LT..T.T...L...A....W.S..QSKAV  M982
T..-.P.S.N.KS.YA.VR..VRLGRWA.V.A.L...YRSTH..DGS.STGTHRT...A.I.L..AD...LT..T.T...L...A....W.S.DK.KAV  FA19
R..-KV.L.K.K..YF.ARN..ALG........I...VSRT.ANESTISVGKFKNF...T.I.I...E...LS..L.T...N........W.Y.GKNDEV  Eagan

         810       820       830       840       850       860       870       880       890       900
TQHGCKGLYYICQQTVHQTKLKPEKSFNQEIGATLHNHLGSLEVSYFKNRYTDLIVGKSEEIRTLTQGDNAGKQRGKGDLGFHNGQDADLTGINILGRLD  4223
..........................................................................................N.........K..  Q8
------------------D.......R.A.IVFKGDF.N..A...N.A.R...AFGY-.T..-----QN.QTSAS..P.YR.A.N.RIA......KI.  B16B6
-------------------.ID.....K.A.IVFKGDF.N..A.W.N.A.R...RGY.AQIK------N..EEA...PAYL.A.S.RI.......KI.  M982
-------------------.ID.....K.A.IVFKGDF.N..A.W.N.A.R...RGY.AQIK------D..EQV..NPAYL.A.S.RI.......KI.  FA19
----------------YVG.F...T.R...F.LA.KGDF.NI.I.H.S.A.RN..AFA-..LSK--------NGT...NY.Y..A.N.K.V.V..TAQ..  Eagan

         910       920       930       940       950       960       970       980       990      1000
LNAVNSRLPYGLYSTLAYNKVDVKGKTLNPTLAG-TNILFDAIQPSRYVVGLGYDAPSQKWGANAIFTHSDAKNPSELLADKNLGNGNIQ-TKQATKAKSTP  4223
..................................-.......................................................-..........  Q8
WHG.WGG..D........RIK..DADIRADRTFV.SY....V......L.....H.DGI..I.TM..Y.K..SVD...GSQA.L...ANAK.A-ASRRTR.  B16B6
WNG.WDK..E.W...F...R.H.RDIKKRADRTDIQSH.................Q.EG...V.GML.Y.K..EIT...GSRA.L...SRN..A-.ARRTR.  M982
WNG.WDK..E.W...F...R.R.RDIKKRADRTDIQSH.............S...Q.EG...V.GML.Y.K..EIT...GSRA.L...SRN..A-.ARRTR.  FA19
F.GLWK.I...W.A.F...Q.K..DQKI.AG..SVSSY..........II.....H..NT..I.TM..Q.K..SQN...GKRA...-.SRDV.S-.RKLTRA  Eagan

        1010      1020      1030      1040      1050      1060      1070
WQTLDLSGYVNIKDNFTLRAGVYNVFNTYYTTWEALRQTAEGAVNQHTGLSQDKHYGRYAAPGRNYQLALEMKF*  4223
..........................................................................*  Q8
.YVT.V...Y...KHL.........LL.YR.V...NV....G........---KNVGV.N.........TFS.....*  B16B6
.YIV.V...YT..KH..........LL.YR.V...NV....G........---KNVGV.N.........TFS.....*  M982
.YIV.V...YTV.KH..........LL.HR.V...NV....A........---KNVGV.N.........TFS.....*  FA19
.HI..V...YMANK.IM..L.I..L..YR.V....V....Q........---QNVGS.T....S....T.T.....*  Eagan
```

Fig. 13

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Tbp2 comparison

```
          10        20        30        40        50        60        70        80        90       100
MKHIPLTTLCVAISAV-LLTACGGS-GGSNPPAPTPIPNASGSGNTGNTGNAGGTDNT-ANAGNTGGT---NSGTGSANTPEPKYQDVPTEKNEKDK-VSSIQEPAM   4223
........................S..F....S......GN...A..A......GGANSG...A....GGA...A.....S.......K.....DE.K.AE-..G.....   Q8
.NN-..VNQAAMVLP.F..S..L.G-.---------------------------------FDLDSVE---.VQDMHSK...EDEKS-QP.SQQD..ENSGA.-   B16B6
.NN-..VNQAAMVLP.F..S..L.G-.---------------------------------FDLDSVD---.EAPRPA-......SS..PQAQ.D-------QG   M982
.NN-..VNQAAMVLP.F..S..L.G-.---------------------------------FDLDSVD---.EAPRPA-......PSK.P.AR.D-------QG   FA19
..SV..ISGGLS----F..S..S.--.---------------------------------FDVDNV--.N.P.---SK.R...DTSNQRK.S-NLKKLFI.SL   Eagan

       110       120       130       140       150       160       170       180       190       200
GYGMALSKINLHNRQDTPLD-EKNIITL--DGKKQVAEG-KKSPLPFS-LDV-ENKLLDGYIAKMNVADKNAIGDRIKKGNKEISDEELAKQIKEAVRKSHEFQQV-   4223
...VE.-.LRNWIP.EQEEH-A.IN-.N--.VV.LEGDL-.HN.PDN.IWQNIK.SKEVQTVYNQEKQNIEDQIK.EN.QRPDKKLDDV.L.AYIEKVLDDRLTELA   Q8
-..F.V-.LPRR.AHFN.KYK..HKP.GSM.W---------.-LQRGEPNSFS.RDE.E----------------K.R.SS.LI-.SKWEDGQSR.VGYTN.T---   B16B6
...F.M-RLKRR.WYP--GAE.SEVK.NES.WEATGLPTKP.E-..KRQKS.I.KVET..D-SDIYSSPYLTPSNHQNG-----.AGNGVN.P.NQATGHEN.---   M982
...F.M-RFKRR.WHPSANPK.DEVK.KND.WEATGLPTEP.K-..LKQQS.ISEVETN.N-S..YTSPYLSQDADS-------.HANG.N.P.NE.TDYKK.----   FA19
.G..K.VAQ..RGNKEPSFLN.DDY.-----------------SY..S.STI.KDVK.KNK---.G..--L..S-.---------..PSTTNPP.K----.HG.---   Eagan

       210       220       230       240       250       260       270       280       290       300
LSSLENKIFHSNDGTTKATTRDLKYVDYGY-YLANDGNYLTVKTDKLWNLGPVGGVFYNGTTTAKELPTQDAVKYKGHWDFMTDVANRRNRFSEVKENS--QA   4223
KPIY.KN.NY.H.KQN..R.......RS..I.RSGYS.---IIPK.IAKT.FD.AL..Q..Q...Q..VSQ-.....T.......-.KKGQS..SPGT-.QRL.   Q8
----------------------..RS..V..-.KN.IDIKNNIV.F--..D.YLY.K.KEPS....-SEKIT...T..YV..AME-KQ..-.GLG-.A..G   B16B6
----------------------..YS.WF.KH.ASEKDPSN.KI.S---.DD.YI..H.EKPSRQ..ASGK.I...V.H.V..TKKGQD.R.IIQP.KK.G   M982
----------------------..YS.WF.KH.KSEVKNENGLVSAKR-..D.YI..H.DKPSRQ..ASE..T...V.H.V..TKQGQK.NDIL.T.KG.G   FA19
----------------------..YS.LY.TPSWSLNDS-.N-.FY-..YY.YA..Y.NK..TN..VNGVA....T...I.ATK.-GK.YPLLSNG.H.--   Eagan

       310       320       330       340       350       360       370       380       390       400
GWYYGASSKD-EYNRLLTKEDSAPDGHSGEYGHSSEFTVNFKEKKLTGKLFSN---LQDRHKGNVTKTERYDIDANIHGNRFRGSATASNK--NDTSK-HPPTSDAN   4223
.DR.S.M.YH-..PS...D.KNK..NYN..........D.SK.S.K.E.S..---I..G...S.N..K........Y..........DTTEASK..-........K   Q8
DK-S..L.AL-.EGV.RNQAE-ASS..TD-F.MT...E.D.SD.TIK.T.YR.NRIT.NNSENKQI..T..T.Q.TL.....K.K.L.AD.--GA.NGS...I..SD   B16B6
DR.S.F.GDGS.EYSNKN-.STLK.D.E.-..FT.NLE.D.GN.......IR.NAS.NNNTNNDKHT.QY.SL..Q.T....N.T...TD.K-ENET.L...V..SS   M982
DK.S.F.GDEG.TTSNR.-DSNLN.K.E.-..FT.N.K.D.NN.......IR.NKVINTAASDGY.-..Y.SL..TLR....S.K.I.TD.PNTGGT.L...VF.SS   FA19
--..RR-.AIP.DID.EN-DSKNG.-I.----LI...SADGGT.....Q.-.YTKRKTNNQPYEKK.L--.....D.YS.....TVKPTE.---.SEE-.....EGT   Eagan

       410       420       430            440                 450       460       470       480
NRLEGGFYGPKGEELAGKFLTNDNKLFGVFGAKRESK------AEEKTE------------------AILDAYALGTFNTSNAT--TFTPFTEKQLDNFGNAKKLV   4223
.S........NA.........................E------.K..------------------..............KPGT.NPA..ANSK.E...........   Q8
S-..................S....VAA.....QKD.KDGENA.GPA.------------------TVI...RIT-----------GEEFKKE.I.S..DV...L   B16B6
S-.S...F..Q....GFR..SD.Q.VAV.GS..TKD.LENGAA.SGS.G-AAASGGAAGTSSENSKLTTV...VE.T-----------LNDKKI.N....S..AQ..   M982
S-.S...F..Q....GFR..SD.G.VAV.GS..TKDST-----.NGNAP-AASSGPGAATMPSETRLTTV...VE.T-----------PDGKEI.N....S..TR..   FA19
--........NA...G....AT..RV....S..ETEETKKEALSK.TLIDGKLITFSTKKTDAKTNATTSTA.NTTTDTTANTI.D--EKN.KTEDISS..E.DY.L   Eagan

       490               500            510         520                           530       540
LGSTVIDLVP------------TDATK--NEFTKDK---PESATNEAGETLMVNDEVSV--------------KTYGKN------FEYLKFGELSIGGSH   4223
..........------------.G...DV...-.E.---.K....K..........I.--------------..........YGRN----..........   Q8
VDGVELS.L.--SE-GNKAA-------------FQHEI.------------------------QNGVKAT-------VCCSNLD.MS..K..KENKD   B16B6
VDGIM.P.L.KDSESGNTQADKGKNGG--T...RKFEHT...DKKD.QAGTQTNGAQTASNTAGDTNGK--T...EVE-VCCSNLN...Y.M.TRKN.K   M982
VDGIM.P.L.--TESGNGQADKGKNGG--TD..YETTYT...DKKDTKAQTGAGGMQTASGTAGVNGGQVGT...KVL VCCSNLN...Y.L..RENNN   FA19
IDKYP.P.L.----------DKNTN---------------------------.FI.SK-----HHTVGN-.R.KVEAVCCSNSDVKS.MYYEDPLKE   Eagan

                                        550       560       570       580       590       600       610
--------------------------------SVFLQGERTATTGEKAVPTTGTAKYLGNWVGYIT-GKDTGTGTGKSFTDAQDVADFTIDPGNKSVSGK   4223
--------------------------------...............E...........-.......S.....NE...I...D...ER...K..   Q8
--------------------------------DM....V..PVSDVA.-R.EAN...R.T.Y...AN.-TSWS.EA-.NQEGGNR.E.DV..ST.KI..T   B16B6
SAMQAGG---------------NSSQADAKTEQVEQ.M..........---D..EI..DQNVV.R.S.Y.H.AN.-TSWS.NA-.DKEGGNR.E..VN.AD.KIT..   M982
SVMQAVK---------------NSSQADAKTKQIEQ.M..........---D.NKI.QEQGIV...F.Y.R.AN.-TSWS.KA-.NATDGNR.K..VN.DR.EIT.T   FA19
KETETETETEKDKEKEKEKDKDKEKQTAATTNTYYQ--..L.H..---PKDDI.K..S...H.S.Y....D..TSYSPS.DKKR.KNA..E.NV..AE.KLT.E   Eagan

       620          630       640       650          660       670       680                     690
LITKGRQDPVFSITGQIAG--NGWTGTASTTKADAGGYKIDSSSTGKSIA--IKDANVTGGFYGPNANEMGGSFT-------------------NADDSKASV   4223
.T.Q.......N.............A..NV..............V--.EN.K.....................................-HDT.......   Q8
.TA.D.TS.A.T..AM.KD--..FS.V.K.---GEN.FAL.PQN..N.HYTH.-E.T.S.....K..I......SFPGNAPEGKQE---------------   B16B6
.TAEN..AQT.T.E.M.Q.---..FE...K.---AES.FDL.QKN.TRTPKAY.T..K.K.......K.E.L..W.AYPGDKQTEKATATSSDG---.SAS.-.T.   M982
.TAEN.SEAT.T.DAM.E.---..FK...K.---GND.FAP.QNNSTVTHMVH.AN.E.Q........E.L..W.AYPGNEQTKNATVESGNG---.SAS.-.T.   FA19
.KRHDTGN.....EANFNNSS.AP....TA.-----NFV..GKNSQNKNTPINITTK.N.A....K.S.L..Y..YNGNSTATNSESSSTVSSSS.SKNAR.A.   Eagan

       700
VFGTKRQQEV-K*   4223
.......E..-.*   Q8
...A....L.Q-*   B16B6
...A....P.Q-*   M982
...A....KL.-.*   FA19
...ARQ.V.TT.*   Eagan
```

Construction of TBP1 Expressi n Plasmid

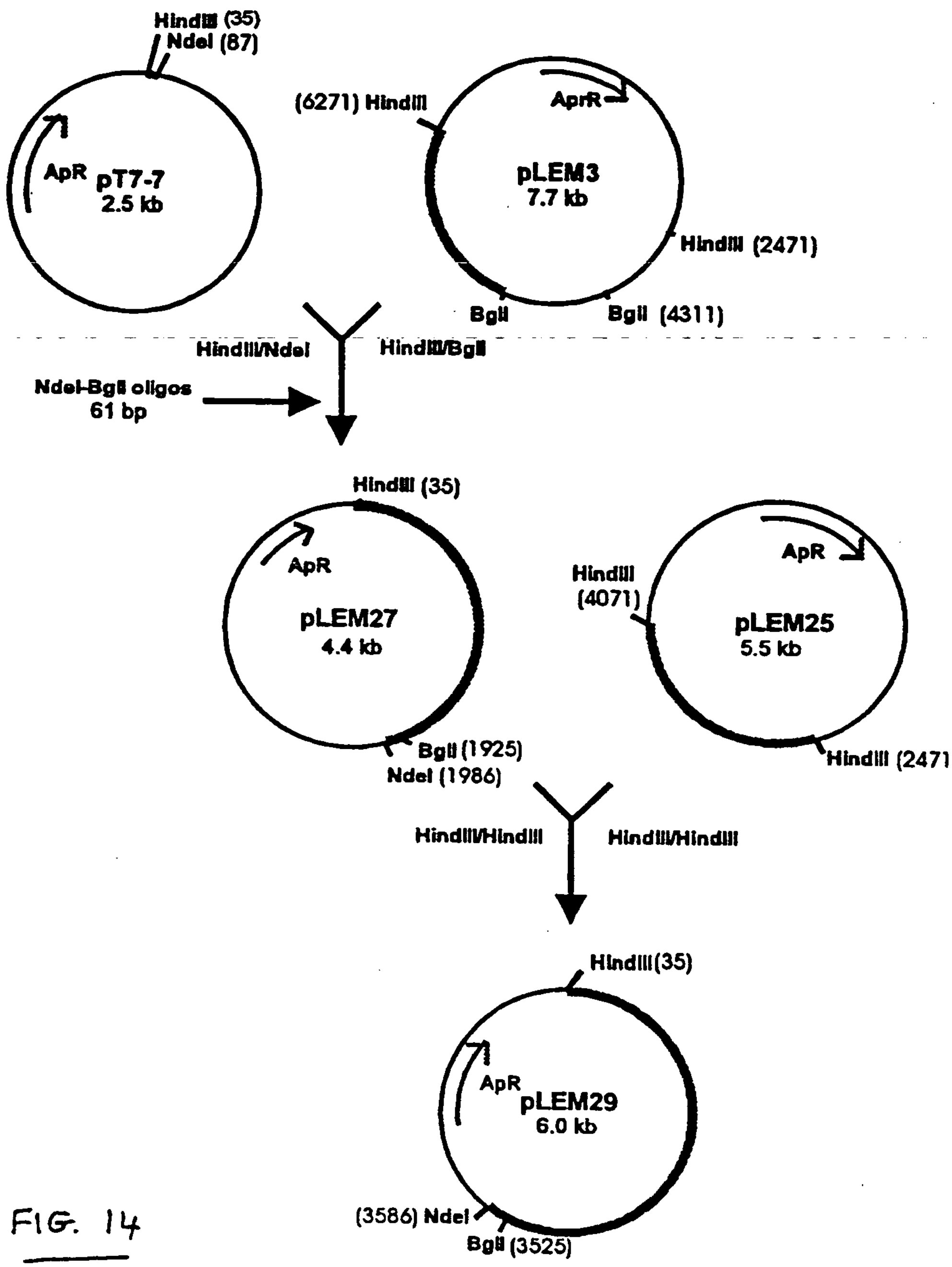

FIG. 14

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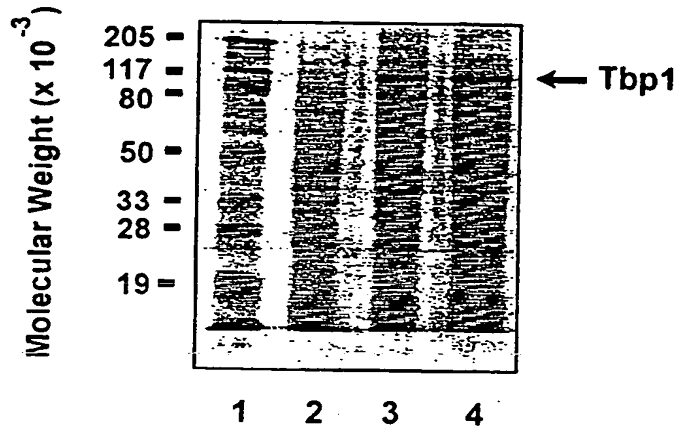
Expression of rTbp1 in *E. coli*



1. Prestained molecular weight markers
2. pLEM29B-1 lysate, non-induced
3. pLEM29B-1 lysate, 1 hr post-induction
4. pLEM29B-1 lysate, 3 hr post-induction

FIG. 15

Purification of Tbp1 from *E. coli*

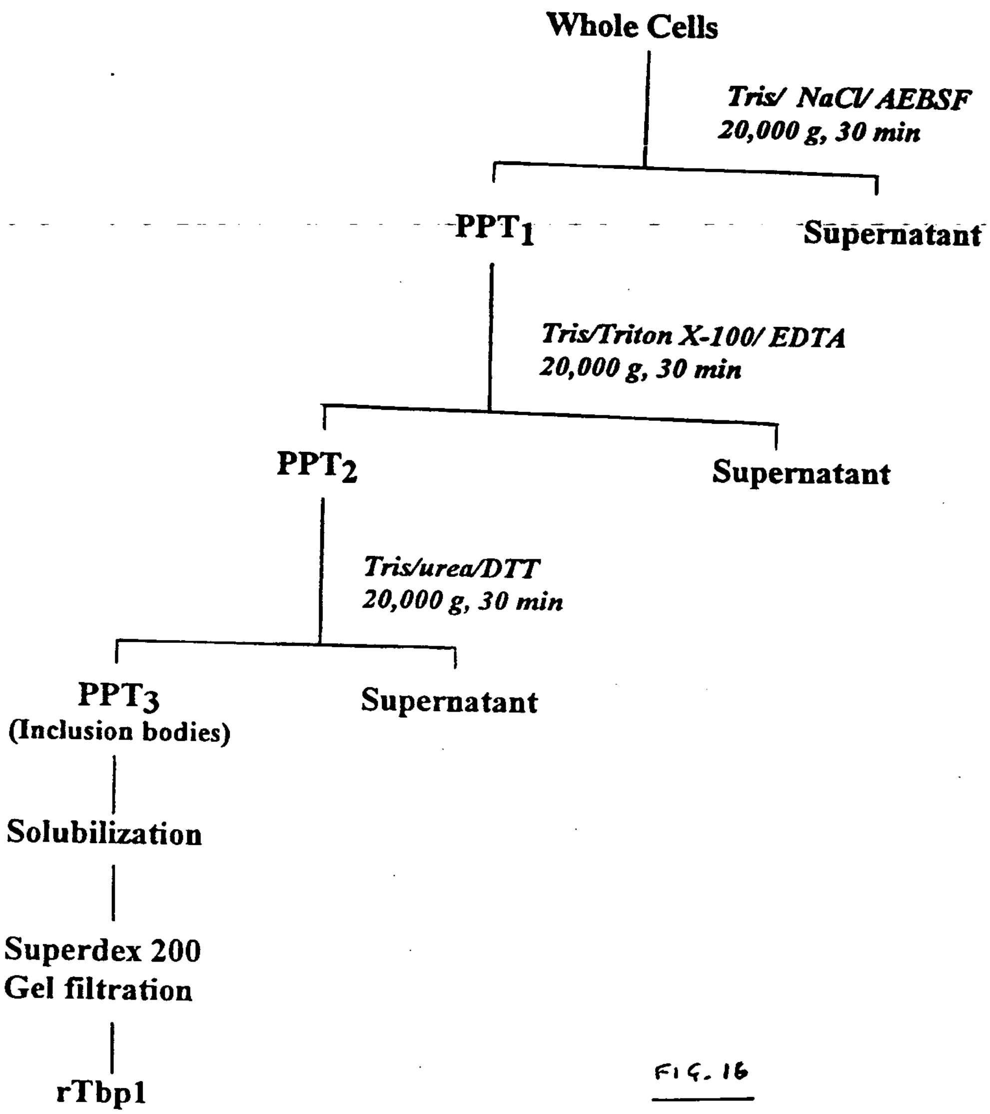
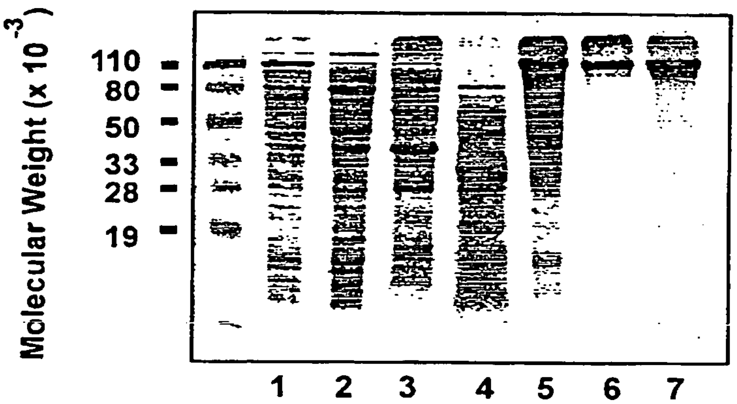


FIG. 16

Purification of rTbp1 from *E. coli*



1. *E. coli* Whole cells
2. Soluble proteins after 50 mM Tris/ NaCl extraction
3. Soluble proteins after Tris/ Triton X-100/ EDTA extraction
4. Soluble proteins after Tris/ urea/ DTT extraction
5. Left-over pellet (rTbp1 inclusion bodies)

6.7. Purified rTbp1

FIG..17

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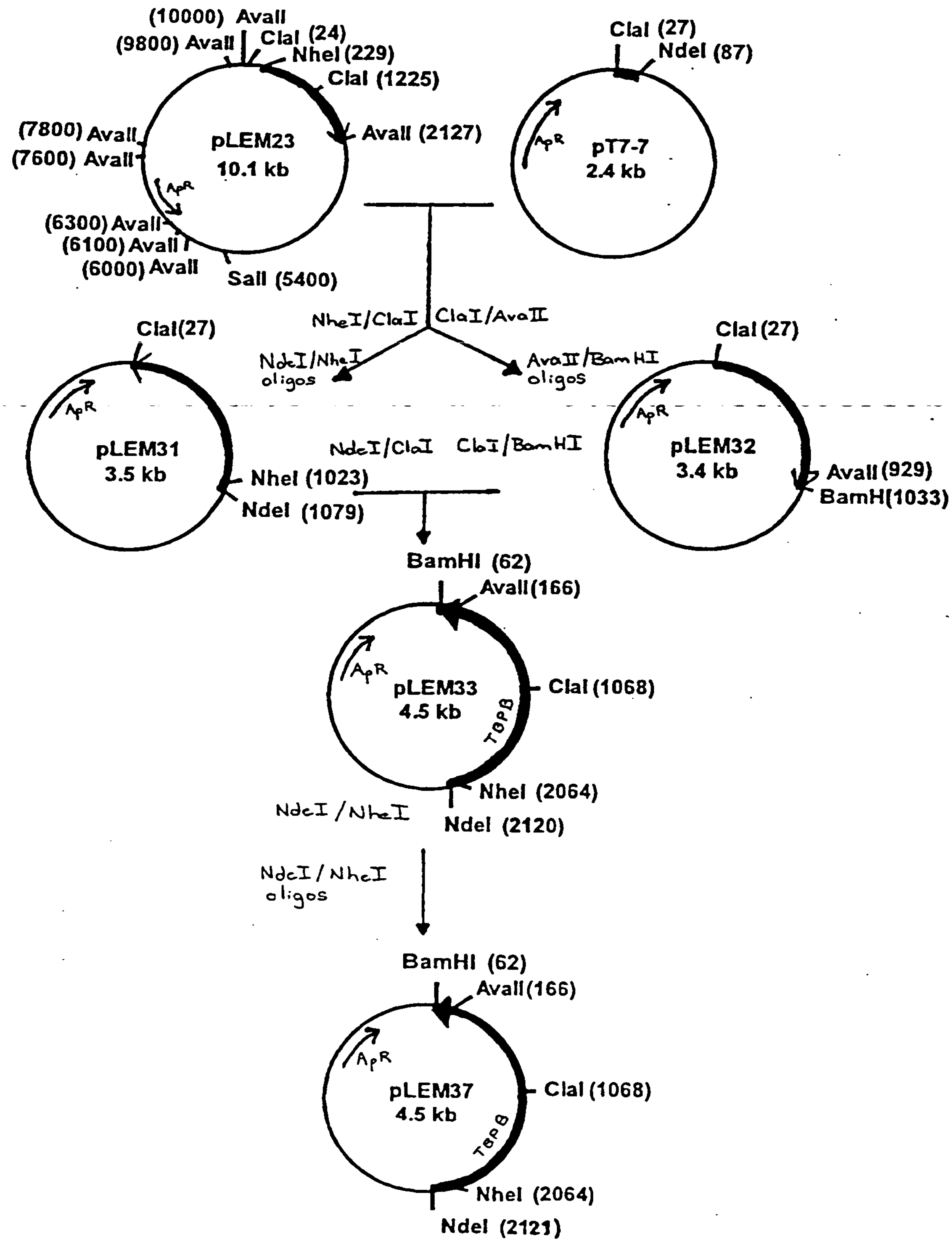
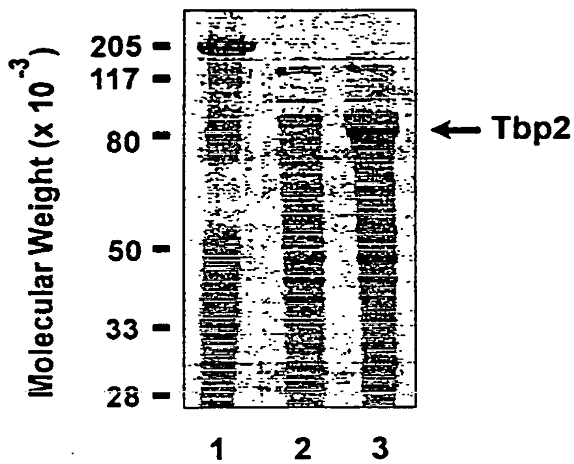
CONSTRUCTION OF TBP2 EXPRESSION PLASMID

FIG 18



Expression of rTbp2 in *E. coli*



1. Prestained molecular weight markers
2. pLEM37B-2 lysate, non-induced
3. pLEM37B-2 lysate, induced

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FIG 20

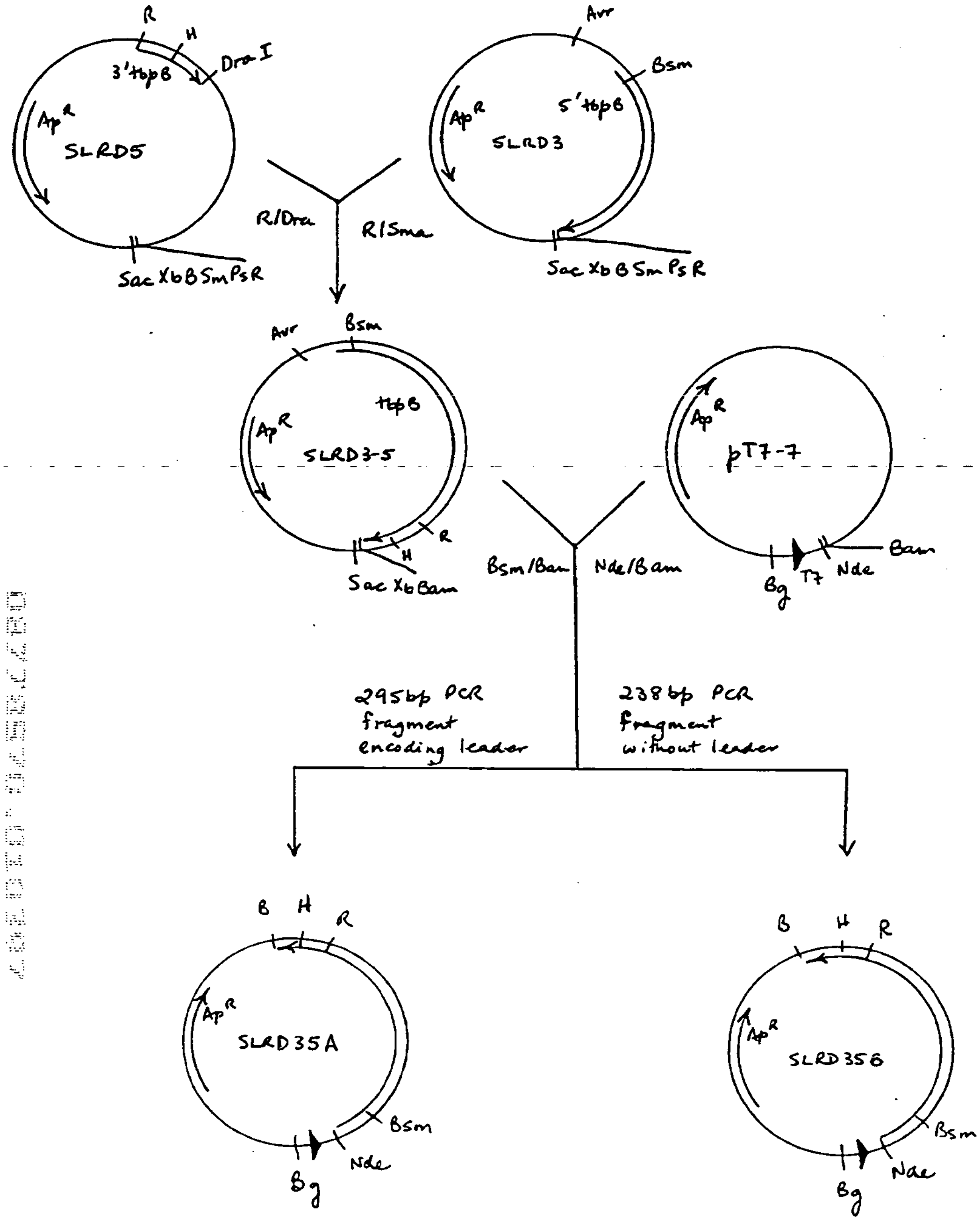

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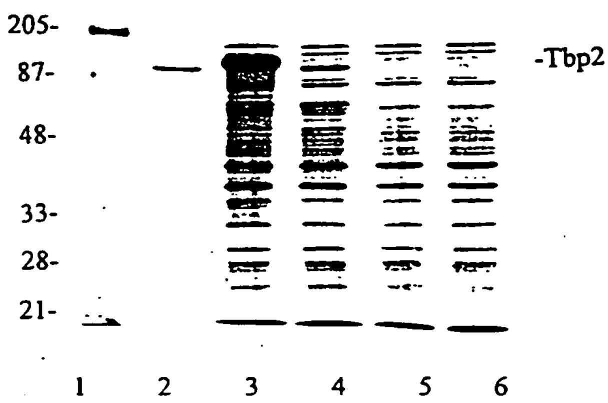
FIG. 21



Expression of Q8 rTbp2 protein in E.coli

1. Prestained molecular weigth marker
2. 4223 rTbp2 protein
3. SLRD35A lysate, 3 hr post-induction
4. SLRD35B lysate, 3 hr post-induction
5. SLRD35A, non-induction
6. SLRD35B, non-induction

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Fig. 22

Purification of Tbp2 from *E. coli*

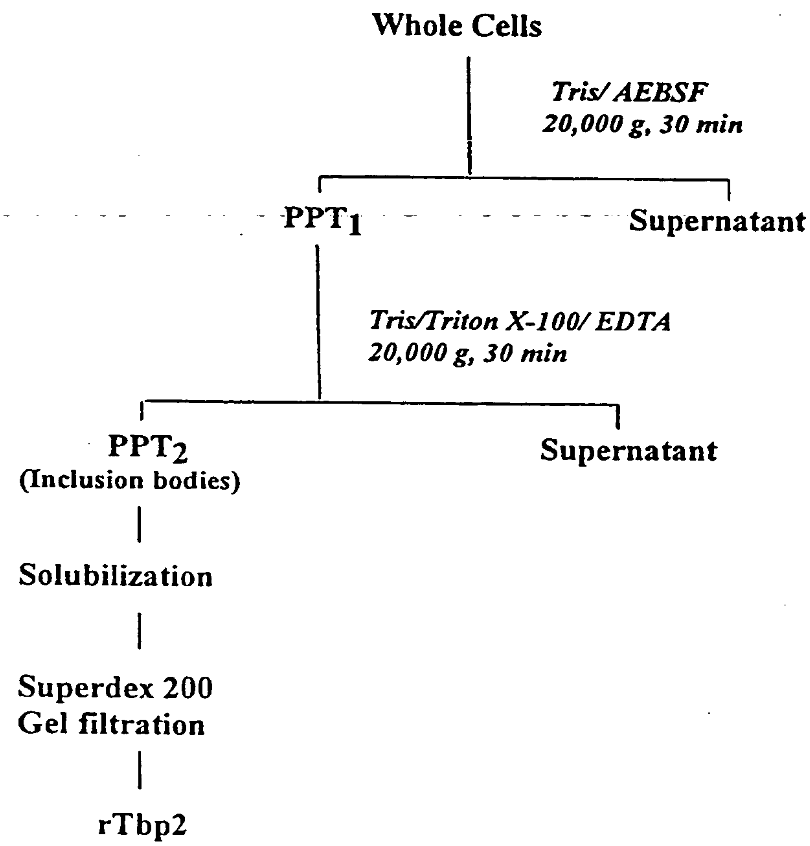

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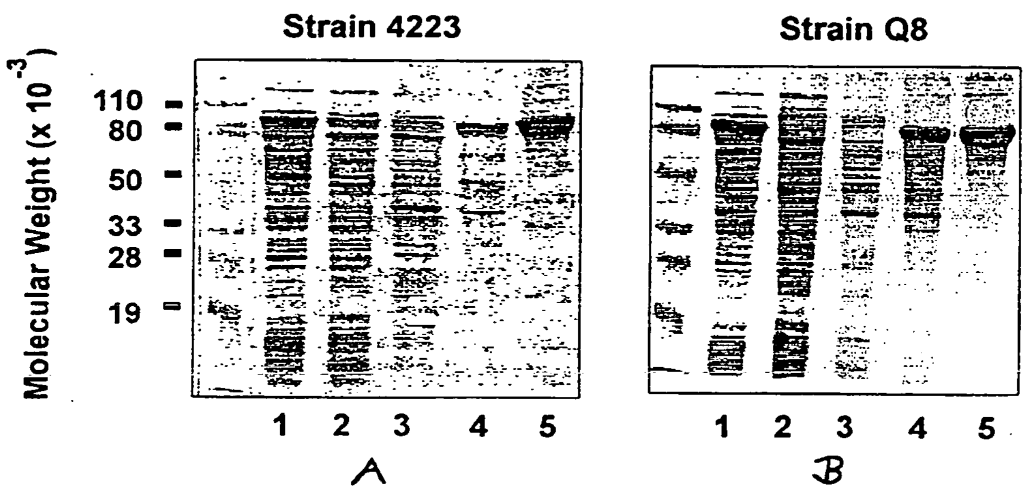
FIG 23

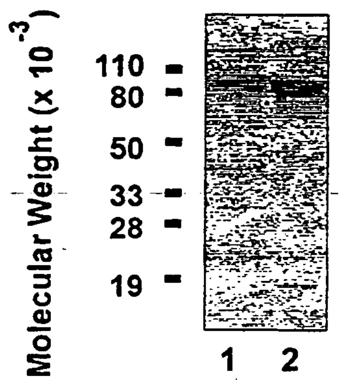
Purification of rTbp2 from *E. coli*



1. *E. coli* Whole cells
2. Soluble proteins after 50 mM Tris extraction
3. Soluble proteins after Tris/ Triton X-100/ EDTA extraction
4. Left-over pellet (rTbp2 inclusion bodies)
5. Purified rTbp2

FIG 24

Binding of Tbp2 to Human Transferrin



1. rTbp2 (strain 4223)
2. rTbp2 (strain Q8)

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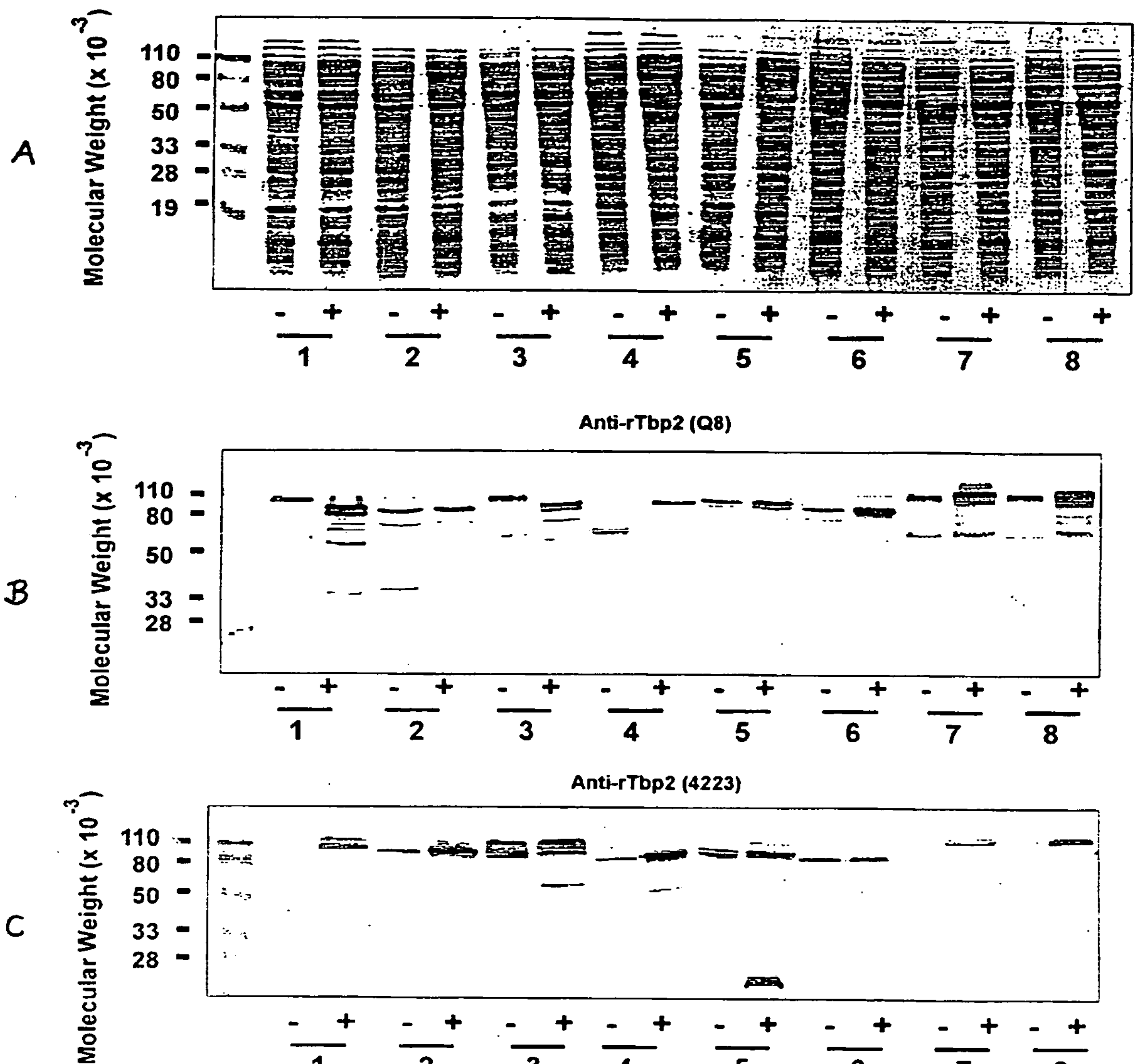
FIG. 25



1. Strain 5191
2. Strain 56
3. Strain 135
4. Strain 4223
5. Strain ATCC25240
6. Strain 585
7. Strain 3
8. Strain 8185